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APPLICATION NUMBER:

209394Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

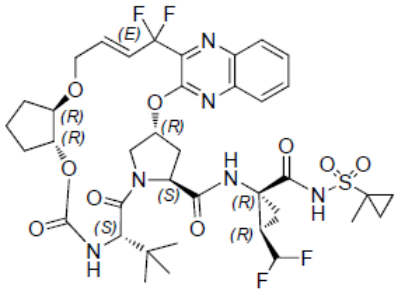
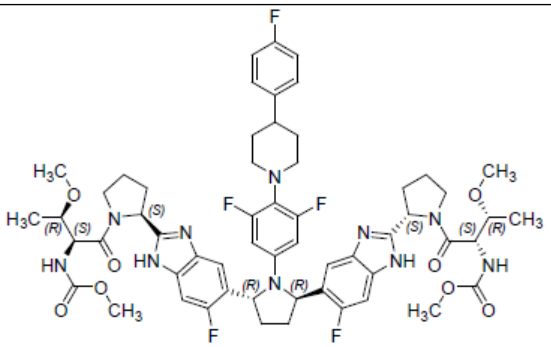
DIVISION OF ANTIVIRAL PRODUCTS
CLINICAL VIROLOGY REVIEW

NDA: 209394 **SDN**: 000 (Addendum to Original NDA Review) **REVIEW COMPLETED:** 7/18/2017
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 209394 **SDN:** 000 (Addendum to Original NDA Review)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

Sponsor: AbbVie, Inc.
1 N. Waukegan Road
North Chicago, IL 60064
Sejal P. Emerson, PharmD
Director, Regulatory Affairs

Initial Complete Submission Dates:
Correspondence Date: 12/14/2016
CDER Receipt Date: 12/14/2016
Assigned Date: 12/15/2016
Review Complete Date: 7/18/2017
PDUFA Date: 8/14/2017

Proprietary Name (tentative)	**Mavyret™** **(fixed dose combination product: glecaprevir/pibrentasvir)**	
Individual Drug Names [class]	glecaprevir (GLE, ABT-493) [NS3/4A protease inhibitor]	pibrentasvir (PIB, ABT-530) [NS5A inhibitor]
Parent IND #s	116169 (GLE), 127416 (GLE/PIB)	116170 (PIB), 127416 (GLE/PIB)
Chemical Names	(3a*R*,7*S*,10*S*,12*R*,21*E*,24a*R*)-7-*tert*-butyl-*N*-{(1*R*,2*R*)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl] cyclopropyl}-20,20-difluoro-5,8-dioxo-2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1*H*,10*H*-9,12-methanocyclopenta[18,19] [1,10,17,3,6]trioxadiazacyclononadecino[11,12-*b*]quinoxaline-10-carboxamide hydrate (IUPAC)	Methyl {(2*S*,3*R*)-1-[(2*S*)-2-{5-[(2*R*,5*R*)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)piperidin-1-yl]phenyl}-5-(6-fluoro-2-{(2*S*)-1-[*N*-(methoxycarbonyl)-*O*-methyl-L-threonyl]pyrrolidin-2-yl}-1*H*-benzimidazol-5-yl)pyrrolidin-2-yl]-6-fluoro-1*H*-benzimidazol-2-yl}pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl}carbamate (IUPAC)
Structures	 • X H_2O **GLECAPREVIR**	 **PIBRENTASVIR**
Molecular Formulas	$C_{38}H_{46}F_4N_6O_9S$ (anhydrate)	$C_{57}H_{65}F_5N_{10}O_8$
Molecular Weights	838.87 (anhydrate)	1113.18

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CLINICAL VIROLOGY REVIEW
NDA: 209394 **SDN**: 000 (Addendum to Original NDA Review) **REVIEW COMPLETED:** 7/18/2017
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

Amendments: none

Related/Supporting Documents: Original NDA 209394 Clinical Virology review; Additional/updated information submitted in the following SDNs: 41 (updated clinical and cell culture data for GT5/6), 42 (response to 5/26/17 labeling comments), 43 (response to 5/26/17 labeling comments, error correction), 46 (response to 6/13/17 labeling comments, and updated GT5/6 data), 47 (late cycle meeting minutes), 48 (PMR/PMC milestones), 49 (response to 7/7/17 labeling comments), 50 (PMC milestones)

Dosage Form and Route of Administration: 100 mg glecaprevir and 40 mg pibrentasvir fixed-dose combination tablet; Oral (3 tablets/day)

Dispensed: Rx x OTC__

Proposed Indication(s): Treatment of patients with chronic HCV genotype (GT) 1, 2, 3, 4, 5 or 6 infection

Abbreviations: EC, effective concentration; GLE, glecaprevir; HCV, hepatitis C virus; GT, genotype; IFNα, interferon alpha; LLOQ, lower limit of quantification; PIB, pibrentasvir; PMC, post-marketing commitment; PMR, post-marketing requirement; RAS, resistance-associated substitution; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virologic response; VF, virologic failure;

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CLINICAL VIROLOGY REVIEW ADDENDUM

This addendum to the Clinical Virology review of NDA 209394 covers the following issues that were addressed following the completion of the original Clinical Virology review:

- 8-week treatment duration for non-cirrhotic patients with HCV genotype (GT) 5 or GT6 infection
- Labeling
- Post-marketing commitments (PMCs)

1. Updated data supporting 8-week duration for non-cirrhotic patients with HCV GT5 or GT6

Since the finalization of the Clinical Virology review of NDA 209394 for glecaprevir/pibrentasvir (GLE/PIB), the review team received new data and had extensive discussions, both internally and with the sponsor, regarding the recommended GLE/PIB treatment duration for non-cirrhotic patients with HCV GT5 or GT6 infection. At the time of completion of the original Clinical Virology review, the review team did not believe the sponsor had sufficient data to support dosing GLE/PIB for 8 weeks for non-cirrhotic patients with these uncommon genotypes; however, GLE/PIB for 12 weeks was well supported. Table 1 (FDA analysis) shows the pooled results for sustained virologic response at 12 weeks post-treatment (SVR12) and virologic failure (VF) that

were available at the time. The overall intent-to-treat SVR12 rates for GT5 and GT6 infected subjects who received GLE/PIB for 8 weeks were 2/2 (100%) and 9/10 (90%; 1 non-VF), with larger numbers of subjects who received GLE/PIB for 12 weeks. No cases of virologic failure occurred with either duration for patients with GT5 or GT6 infection.

Table 1. Pooled SVR12 and virologic failure results for NS3/4A PI-naïve and NS5A inhibitor-naïve subjects (i.e., excluding MAGELLAN-1/M15-410) who received GLE/PIB to-be-marketed dose levels in Phase 2b/3 trials. For non-cirrhotic subjects, yellow rows show data for 8W regimens (all from the SURVEYOR-2 trial), and green rows show data for 12W regimens.

HCV GT	Cirrhosis	Tx History	Regimen	SVR12	VF Rate	On-Tx VF	Relapse	Non-VF
GT5	N	Naïve	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (22/22)	0% (0/22)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (2/2)	0% (0/2)	0	0	0
GT6	N	Naïve	GLE/PIB 8W	88% (7/8)	0% (0/8)	0	0	1
			GLE/PIB 12W	100% (27/27)	0% (0/27)	0	0	0
		IFN- or P/R-exp	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (4/4)	0% (0/4)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	1/1	0/1	0	0	0

Newly available clinical efficacy data are summarized in Table 2 (adapted from SDN 46 response document, pg. 2). First, the sponsor confirmed that the one GT6 subject (2811) who failed GLE/PIB for 8 weeks for non-virologic reasons (lost to follow-up) in SURVEYOR-2 in fact achieved SVR12 and SVR24. In addition, 4 other GT6 infected subjects from the ENDURANCE-4 and EXPEDITION-2 trials achieved SVR12 and SVR24 with GLE/PIB durations of 8 weeks or less. Thus, the pooled SVR12 rate for GT6 infected patients who received GLE/PIB dosed for 8 weeks or less is 14/14 (100%).

In addition, the sponsor recently initiated a new clinical trial, M16-126, which is studying GLE/PIB in a larger number of subjects with HCV GT5 or GT6 infection. As of 6/13/17, there were 20 non-cirrhotic subjects (5 GT5, 15 GT6) assigned to 8 weeks of GLE/PIB treatment. Four subjects (1 GT5, 3 GT6) have reached the SVR4 analysis timepoint, and all achieved SVR4 (Table 2). The other 16 subjects have HCV RNA data available through Treatment Weeks 4 or 8, with HCV RNA <LLOQ (n=1, GT6 subject at Week 4) or not detected (n=15) through the last available timepoint; no subjects have experienced virologic failure.

Table 2. Newly available efficacy results for HCV GT5 or GT6 infected subjects who received GLE/PIB for ≤8 weeks.

Subject	GT Subtype	Study	SVR4	SVR12	SVR24	Study Description/Comments
2811	6a/b	SURVEYOR-2-Part-4 (M14-868)	Yes	Yes*	Yes	*Previously counted as lost-to-follow-up
200402	6a	ENDURANCE-4 (M13-583)	Yes	Yes	Yes	Received 19 days of tx
110207	6u	EXPEDITION-2 (M14-730)	Yes	Yes	Yes	Study in HIV-1 coinfected subjects
302202	6e	EXPEDITION-2 (M14-730)	Yes	Yes	Yes	Study in HIV-1 coinfected subjects
302203	6n	EXPEDITION-2 (M14-730)	Yes	Yes	Yes	Study in HIV-1 coinfected subjects
30002	6a	M16-126	Yes	Ongoing		Study in GT5 or GT6-infected subjects
30001	6a	M16-126	Yes	Ongoing		Study in GT5 or GT6-infected subjects
30004	6a	M16-126	Yes	Ongoing		Study in GT5 or GT6-infected subjects
50002	5a	M16-126	Yes	Ongoing		Study in GT5 or GT6-infected subjects

To confirm durability of SVR12 response, the Division requested that the sponsor provide an update on available SVR24 results from HCV GT5 or GT6 infected subjects. The sponsor reported on 6/13/17 that all subjects infected with GT5 or GT6 without cirrhosis treated with GLE/PIB for 8 or 12 weeks whose SVR12 data were available and included in the NDA submission at filing achieved SVR24 (11/11 and 56/56 for 8 and 12 week durations, respectively), with no subjects experiencing late relapse.

The sponsor also provided new cell culture data addressing the impact of a GT5a NS3 D168E substitution on the anti-HCV activity of GLE (Table 3; adapted from SDN 41 report, pg. 1). As noted in the original Clinical Virology review, an NS3 D168E polymorphism was detected in 42% (13/31) of HCV GT5a infected subjects with available NS3/4A sequence analysis data. Various amino acid substitutions at NS3 position D168, alone or in combination with other NS3 substitutions can reduce GLE activity in multiple HCV genotypes/subtypes, so there is a theoretical concern that the D168E polymorphism may reduce GLE/PIB efficacy for GT5. Nevertheless, all 13 HCV GT5 infected subjects with the D168E polymorphism (1 cirrhotic, 12 non-cirrhotic) achieved SVR12; all received GLE/PIB for 12 weeks. As shown in Table 3, introduction of D168E into a GT5a replicon conferred a relatively modest 4.2-fold reduction in GLE activity, but activity remained in the sub-nanomolar level and comparable to the activity against wild-type HCV replicons of other genotypes. Of note, wild-type HCV GT3 similarly has an NS3 D168Q change relative to GT1, and GLE/PIB for 8-weeks is well supported for treatment-naïve, non-cirrhotic GT3-infected patients, with an SVR12 rate of 94.9% (149/157) and relapse rate of 3% (5/150).

Table 3. Anti-HCV activity of GLE against HCV GT5a replicon with D168E substitution.

Replicon	n	Mean glecaprevir EC_{50} value +/- SD (nM)	Fold-change in EC_{50} value
GT5a-wt	6	0.096 ± 0.033	--
GT5a-D168E	6	0.409 ± 0.134	4.2

Finally, as noted above, clinical trial M16-126 is ongoing and will provide additional data addressing the efficacy of GLE/PIB for 8 weeks in non-cirrhotic GT5 or GT6 infected patients (as well as GLE/PIB for 12 weeks in cirrhotic patients). The design of M16-126 is summarized in Figure 1 (M16-126 protocol pg. 23). The sponsor has agreed to submit a final study report along with (b) (4) drug resistance datasets from M16-126 as a PMC.

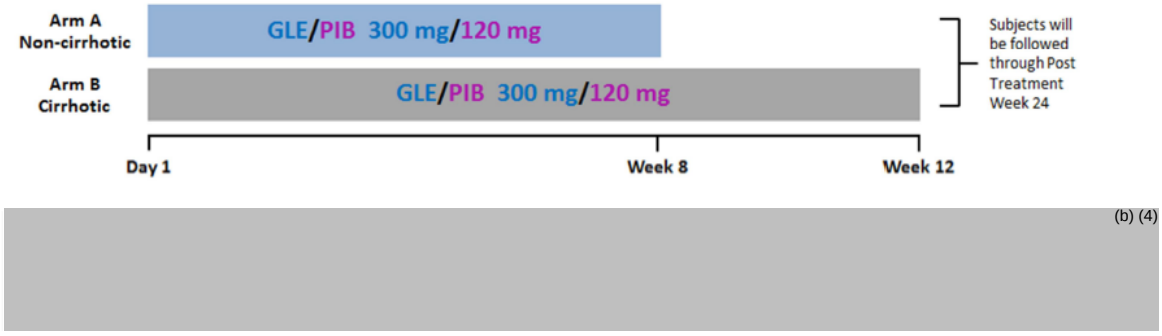

Figure 1. Study design schematic for ongoing trial M16-126 in subjects with HCV GT5 or GT6 infection.

Based on the totality of available data, as well as the expectation of additional confirmatory data from ongoing clinical trial M16-126, the review team concluded, and this reviewer agrees, that the 8-week treatment duration is appropriate for non-cirrhotic patients with GT5 or GT6 infection. This duration is well supported for patients with HCV GT1-4 infection, including patients with GT1a or GT3 (treatment-naïve only for GT3) who are traditionally the most difficult to treat with DAA-based regimens. Although some extrapolation is necessary to

extend the 8-week duration to GT5 and GT6, particularly for GT5 infected patients or treatment-experienced (IFNα, RBV and/or SOF) GT5 or GT6 patients, there is no evidence from available nonclinical and clinical data that patients with GT5 or GT6 respond sub-optimally to GLE/PIB relative to other HCV genotypes. For transparency and to help justify the 8-week duration in labeling, Section 14 will show available GT5 and GT6 SVR12 data for both 8-week and 12-week GLE/PIB durations. Some extrapolation is acceptable for GT5 and GT6 given they are the least common genotypes in the U.S. and worldwide (except for the rare GT7 from a few documented cases), and represent ≤1% of the U.S. HCV infected population (Gower et al., 2014; Messina et al., 2015). On a practical level, this will allow for a simple, 8-week GLE/PIB duration for all treatment-naïve, non-cirrhotic patients regardless of HCV genotype.

2. Final labeling for 12.4 Microbiology

The final agreed text for Section 12.4 Microbiology is pasted below (updated label submitted in SDN 49; with minor typographical correction shown below communicated to sponsor). One significant change from earlier draft versions of the label was the removal (b) (4) and replacement with observed EC_{50} value ranges, as we did not believe it is was necessary to include (b) (4)

12.4 Microbiology

Mechanism of Action

Glecaprevir

Glecaprevir is an inhibitor of the HCV NS3/4A protease, which is necessary for the proteolytic cleavage of the HCV encoded polyprotein (into mature forms of the NS3, NS4A, NS4B, NS5A, and NS5B proteins) and is essential for viral replication. In a biochemical assay, glecaprevir inhibited the proteolytic activity of recombinant NS3/4A enzymes from clinical isolates of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a with IC_{50} values ranging from 3.5 to 11.3 nM.

Pibrentasvir

Pibrentasvir is an inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. The mechanism of action of pibrentasvir has been characterized based on cell culture antiviral activity and drug resistance mapping studies.

Antiviral Activity

In HCV replicon assays, glecaprevir had median EC_{50} values of 0.08-4.6 nM against laboratory and clinical isolates from subtypes 1a, 1b, 2a, 2b, 3a, 4a, 4d, 5a, and 6a. Pibrentasvir had median EC_{50} values of 0.5-4.3 pM against laboratory and clinical isolates from subtypes 1a, 1b, 2a, 2b, 3a, 4a, 4b, 4d, 5a, 6a, 6e and 6p.

Combination Antiviral Activity

Evaluation of combination of glecaprevir and pibrentasvir showed no antagonism in antiviral activity in HCV genotype 1 replicon cell culture assays.

Resistance

In Cell Culture

Selection of HCV genotype 1a, 1b, 2a, 3a, 4a or 6a replicons for reduced susceptibility to glecaprevir resulted in the emergence of amino acid substitutions most commonly at NS3 positions A156 or D/Q168. Individual substitutions at NS3 amino acid position A156 introduced into HCV replicons by site-directed mutagenesis generally caused the greatest reductions (>100-fold) in susceptibility to glecaprevir. Individual substitutions at NS3 position D/Q168 had varying effects on glecaprevir susceptibility depending on HCV genotype/subtype and specific amino acid change, with the greatest reductions (>30-fold) observed in genotypes 1a (D168F/Y), 3a (Q168R) and 6a (D168A/G/H/V/Y). Combinations of NS3 Y56H plus D/Q168 substitutions resulted in greater reductions in glecaprevir susceptibility. An NS3 Q80R substitution in genotype 3a caused a 21-fold reduction in glecaprevir susceptibility, while Q80 substitutions in genotypes 1a and 1b (including genotype 1a Q80K) did not reduce glecaprevir susceptibility. Individual amino acid substitutions associated with resistance to other HCV protease inhibitors at positions 36, 43, 54, 55, 56, 155, 166, or 170 in NS3 generally did not reduce susceptibility to glecaprevir.

Selection of HCV genotype 1a, 2a or 3a replicons for reduced susceptibility to pibrentasvir resulted in the emergence of amino acid substitutions at known NS5A inhibitor resistance-associated positions, including Q30D/deletion, Y93D/H/N or H58D +Y93H in genotype 1a replicons, F28S + M31I or P29S + K30G in genotype 2a replicons, and Y93H in genotype 3a replicons. The majority of individual amino acid substitutions associated with resistance to other HCV NS5A inhibitors at positions 24, 28, 30, 31, 58, 92, or 93 in NS5A did not reduce susceptibility to pibrentasvir. Individual NS5A amino acid substitutions that reduced susceptibility to pibrentasvir include M28G or Q30D in a genotype 1a replicon (244- and 94-fold, respectively), and P32-deletion in a genotype 1b replicon (1,036-fold). Some combinations of two or more NS5A inhibitor resistance-associated amino acid substitutions may result in greater reductions in pibrentasvir susceptibility.

In Clinical Studies

Studies in Treatment-Naïve and Peginterferon, Ribavirin and/or Sofosbuvir Treatment-Experienced Subjects with or without Cirrhosis

In pooled analyses of NS3/4A PI- and NS5A inhibitor-naïve subjects who received MAVYRET for 8, 12, or 16 weeks in Phase 2 and 3 clinical studies, treatment-emergent resistance analyses were conducted for 22 subjects who experienced virologic failure (2 with genotype 1, 2 with genotype 2, 18 with genotype 3 infection). No subjects with HCV genotype 4, 5 or 6 infection experienced virologic failure.

Among the two genotype 1-infected subjects who experienced virologic failure, both subjects had a subtype 1a infection. One subject had treatment-emergent substitutions A156V in NS3, and Q30R, L31M and H58D in NS5A (Q30R and L31M were also detected at a low frequency at baseline). One subject had treatment-emergent Q30R and H58D (while Y93N was present at baseline and post-treatment) in NS5A.

Among the two genotype 2-infected subjects who experienced virologic failure, both subjects had a subtype 2a infection, and no treatment-emergent substitutions were observed in NS3 or NS5A.

Among the 18 genotype 3-infected subjects who experienced virologic failure, treatment-emergent NS3 substitutions Y56H/N, Q80K/R, A156G, or Q168L/R were observed in 11 subjects. A166S or Q168R were present at baseline and post-treatment in 5 subjects. Treatment-emergent NS5A substitutions

M28G, A30G/K, L31F, P58T, or Y93H were observed in 16 subjects, and 13 subjects had A30K (n=9) or Y93H (n=5) at baseline and post-treatment.

Studies in Subjects with or without Cirrhosis Who Were Treatment-Experienced to NS3/4A Protease and/or NS5A Inhibitors

Treatment-emergent resistance analyses were conducted for 11 HCV genotype 1 infected subjects (10 genotype 1a, 1 genotype 1b) with prior NS3/4A PI or NS5A inhibitor treatment experience who experienced virologic failure with MAVYRET with or without ribavirin in the MAGELLAN-1 study. Treatment-emergent NS3 substitutions V36A/M, Y56H, R155K/T, A156G/T/V, or D168A/T were observed in 73% (8/11) of subjects. Nine of 10 subjects (90%, not including one subject missing NS5A data at failure) had treatment-emergent NS5A substitutions M28A/G (or L28M for genotype 1b), P29Q/R, Q30K/R, H58D or Y93H/N. All 11 subjects also had NS5A inhibitor resistance-associated substitutions detected at baseline, and 7/11 had NS3 PI resistance-associated substitutions detected at baseline (see Cross-Resistance for the effect of baseline resistance-associated substitutions on treatment response for NS3/4A PI or NS5A inhibitor treatment-experienced patients).

Effect of Baseline HCV Amino Acid Polymorphisms on Treatment Response (NS3/4A PI- and NS5A Inhibitor-Naïve Subjects)

A pooled analysis of NS3/4A PI- and NS5A inhibitor-naïve subjects who received MAVYRET in the Phase 2 and Phase 3 clinical studies was conducted to identify the HCV subtypes represented and explore the association between baseline amino acid polymorphisms and treatment outcome . Baseline polymorphisms relative to a subtype-specific reference sequence at resistance-associated amino acid positions 155, 156, and 168 in NS3, and 24, 28, 30, 31, 58, 92, and 93 in NS5A were evaluated at a 15% detection threshold by next-generation sequencing. Among subjects who received MAVYRET for 8-, 12-, or 16 weeks, baseline polymorphisms in NS3 were detected in 1% (9/845), 1% (3/398), 2% (10/613), 1% (2/164), 42% (13/31), and 3% (1/34) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively. No baseline polymorphisms were detected at NS3 amino acid position 156 across all genotypes. Baseline polymorphisms in NS5A were detected in 27% (225/841), 80% (331/415), 22% (136/615), 50% (80/161), 13% (4/31), and 54% (20/37) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively.

Genotype 1, 2, 4, 5, and 6: Baseline HCV polymorphisms in genotypes 1, 2, 4, 5 and 6 had no impact on treatment outcome.

Genotype 3: Among treatment-naïve, genotype 3-infected subjects without cirrhosis who received MAVYRET for 8 weeks, an NS5A A30K polymorphism was detected in 10% (18/181) of subjects, of whom 78% (14/18) achieved SVR12. Insufficient data are available to characterize the impact of the A30K polymorphism in genotype 3-infected subjects with cirrhosis (n=1, SVR12) or prior treatment experience (n=1, relapse) who received the recommended MAVYRET regimens. All genotype 3-infected subjects (100%, 16/16) with Y93H in NS5A at baseline who received the recommended MAVYRET regimens achieved SVR12.

Cross-resistance

Based on resistance patterns observed in cell culture replicon studies and HCV-infected subjects, cross-resistance is possible between glecaprevir and other HCV NS3/4A PIs, and between pibrentasvir and

other HCV NS5A inhibitors. Cross-resistance is not expected between MAVYRET and sofosbuvir, pegylated interferon or ribavirin.

In the MAGELLAN-1 study, HCV genotype 1-infected subjects who had failed prior treatment with NS3/4A protease and/or NS5A inhibitors were treated with MAVYRET for 12 or 16 weeks. Baseline sequences were analyzed by next generation sequencing at a 15% detection threshold.

Among 23 NS3/4A PI-experienced/NS5A inhibitor-naïve subjects who received MAVYRET for 12 weeks in MAGELLAN-1 (excluding 2 non-virologic failure subjects), 2 subjects each had baseline NS3 R155K or D168E/V substitutions; all 23 subjects achieved SVR12.

Among NS5A inhibitor-experienced/PI-naïve subjects who received MAVYRET for 16 weeks, baseline NS5A resistance-associated substitutions [R30Q (n=1), Y93H/N (n=5), M28A+Q30R (n=1), Q30H+Y93H (n=1), Q30R+L31M (n=2), L31M+H58P (n=1)], were detected in 73% (11/15) of subjects with available data, of whom 91% (10/11) achieved SVR12. The non-SVR12 subject experienced on-treatment virologic failure and had a genotype 1a infection with baseline NS5A Q30R and L31M substitutions.

Persistence of Resistance-Associated Substitutions

Data on the persistence of glecaprevir and pibrentasvir resistance-associated substitutions are not available. NS5A resistance-associated substitutions observed in patients treated with other NS5A inhibitors have been found to persist for longer than 1 year. In patients treated with other NS3/4A PIs, viral populations with NS3 resistance-associated substitutions have been found to decline in some patients through post-treatment weeks 24 and 48. The long-term clinical impact of the emergence or persistence of virus containing glecaprevir or pibrentasvir resistance-associated substitutions is unknown.

3. Post-marketing commitments (PMCs)

The sponsor has agreed to the following virology-related PMCs. Note that the requested site-directed mutant phenotype data are based on Clinical Virology reviews by both Patrick Harrington, Ph.D. and Eric Donaldson, Ph.D.

Submit the final report and datasets, including drug resistance datasets, for the ongoing clinical trial M16-126, evaluating glecaprevir/pibrentasvir in patients with HCV genotype 5 or 6 infection.

Protocol Submission: 11/17/2016
Study/Trial Completion (SVR12 database lock): 08/01/2018
Final SVR12 Report Submission: 03/31/2019
(b) (4)

Submit the final clinical study report and datasets for the ongoing trial M14-730 (EXPEDITION-2) to provide additional efficacy and safety data in HIV/HCV coinfected subjects receiving glecaprevir and pibrentasvir.

Final Protocol (Amendment 3) Submission: 07/22/2016
Study/Trial Completion (SVR12 database lock): 04/03/2017
Final SVR12 Report Submission: 10/31/2017

Conduct a study evaluating the efficacy of glecaprevir/pibrentasvir in HCV genotype 1 infected subjects with prior treatment experience with an NS5A inhibitor plus sofosbuvir regimen. The sponsor will address this PMC with data from ongoing trial (b) (4)

Final (Amended) Protocol Submission: 05/30/2017
Study/Trial Completion (SVR12 database lock): 12/31/2018
Final SVR12 Report Submission: 06/30/2019

Conduct a study to characterize the phenotypic effect of the following individual NS3/4A or NS5A substitutions on the cell culture anti-HCV activity of glecaprevir or pibrentasvir, respectively: genotype 1a NS3_I18V, NS3_N77S, NS3_V116A, NS3_I354V and NS4A_V23A, genotype 3a NS3_I366V, and genotype 1a NS5A_A61T.
Final Report Submission: 03/31/2018

4. CONCLUSIONS

NDA 209394 for glecaprevir/pibrentasvir, including the finalized labeling and PMCs, is approvable from a Clinical Virology perspective.

5. ADMINISTRATIVE

5.1 Reviewer's Signature

_____
Patrick R. Harrington, Ph.D.
Senior Clinical Virology Reviewer, Division of Antiviral Products

5.2 Concurrence

_____
Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antiviral Products

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

/s/

PATRICK R HARRINGTON
07/18/2017

JULIAN J O REAR
07/18/2017

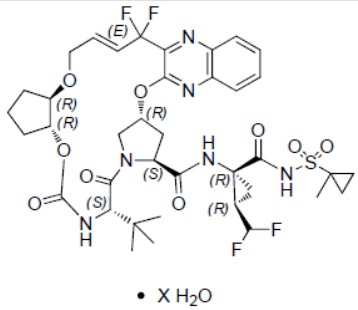
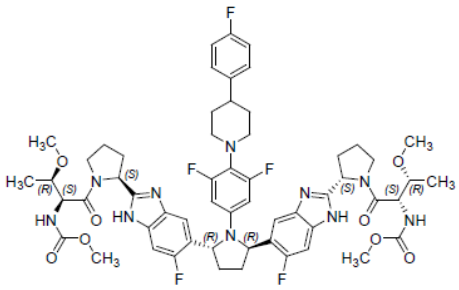
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 209394 **SDN** 003 **DATE REVIEWED:** 04/07/2017

NDA#: 209394 **Serial #: 001**

Reviewer's Name: Eric F. Donaldson, Ph.D.

Sponsor: AbbVie, Inc.
1 N. Waukegan Road
North Chicago, IL 60064
Sejal P. Emerson, PharmD
Director, Regulatory Affairs

Initial Complete Submission Dates:
Correspondence Date: December 14, 2016
CDER Receipt Date: December 14, 2016
Assigned Date: December 15, 2016
Review Complete Date: May 11, 2017
PDUFA Date: August 14, 2017

Proprietary Name	(b) (4) **(fixed dose combination product: glecaprevir/pibrentasvir)**	
Individual Drug Names [class]	glecaprevir (GLE, ABT-493) [NS3/4A protease inhibitor]	pibrentasvir (PIB, ABT-530) [NS5A inhibitor]
Parent IND #s	116169 (GLE), 127416 (GLE/PIB)	116170 (PIB), 127416 (GLE/PIB)
Chemical Names	(3a*R*,7*S*,10*S*,12*R*,21*E*,24a*R*)-7-*tert*-butyl-*N*-{(1*R*,2*R*)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl] cyclopropyl}-20,20-difluoro-5,8-dioxo-2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1*H*,10*H*-9,12-methanocyclopenta[18,19] [1,10,17,3,6]trioxadiazacyclononadecino[11,1-*b*]quinoxaline-10-carboxamide hydrate (IUPAC)	Methyl {(2*S*,3*R*)-1-[(2*S*)-2-{5-[(2*R*,5*R*)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)piperidin-1-yl]phenyl}-5-(6-fluoro-2-{(2*S*)-1-[*N*-(methoxycarbonyl)-*O*-methyl-L-threonyl]pyrrolidin-2-yl}-1*H*-benzimidazol-5-yl)pyrrolidin-2-yl]-6-fluoro-1*H*-benzimidazol-2-yl}pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl}carbamate (IUPAC)
Structures	 • X H_2O **GLECAPREVIR**	 **PIBRENTASVIR**
Molecular Formulas	$C_{38}H_{46}F_4N_6O_9S$ (anhydrate)	$C_{57}H_{65}F_5N_{10}O_8$
Molecular Weights	838.87 (anhydrate)	1113.18

Amendments:

SDN	Date Submitted	Date Received	Date Assigned
001	12/14/2016	12/14/2016	12/15/2016
003	12/20/2016	12/21/2016	12/21/2016
007	01/27/2017	01/27/2017	01/27/2017
010	02/22/2017	02/22/2017	02/22/2017

Related/Supporting Documents: IND116169 (GLE), IND116170 (PIB), and IND127416 (GLE/PIB)

Dosage Form and Route of Administration: 100 mg glecaprevir and 40 mg pibrentasvir fixed-dose combination tablet; Oral (3 tablets/day)

Dispensed: Rx

Proposed Indication(s): Treatment of patients with chronic HCV genotype (GT) 1, 2, 3, 4, 5 or 6 infection

Abbreviations: BOC, boceprevir; CC, cytotoxicity concentration; CSR, clinical study report; DAA, direct-acting antiviral agent; DBV, dasabuvir; EC, effective concentration; EOT, end of treatment; ESRD, end-stage renal disease; FDC, fixed-dose combination; GLE, glecaprevir; GLP, good laboratory practice; GT, genotype; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV(-1), human immunodeficiency virus (type 1); IC, inhibitory concentration; IFN(α), interferon (alfa); ITT, intent-to-teat; LiPA, line-probe assay; LLOQ, lower limit of quantification; NGS, next generation sequencing; OMB, ombitasvir; Peg-IFNα, pegylated interferon alfa; PI, protease inhibitor; PIB, pibrentasvir; PK, pharmacokinetic; PM, polymorphism; P/R, pegylated interferon alfa plus ribavirin; P/R/S, regimens containing interferon, pegylated interferon, ribavirin, and/or sofosbuvir; PTV, paritaprevir; RAS, resistance-associated substitution; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SMV, simeprevir; SOF, sofosbuvir; SVR, sustained virologic response; TE, treatment-experienced; TN, treatment-naïve; TND, target not detected; TVR, telaprevir; UTR, untranslated region; VF, virologic failure;

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EXECUTIVE SUMMARY

The sponsor submitted this original New Drug Application (NDA) for the fixed-dose combination (FDC) of glecaprevir (GLE, ABT-493) and pibrentasvir (PIB, ABT-530) (GLE/PIB 300/120 mg) to be used in the treatment of chronic hepatitis C virus (HCV) infection. GLE/PIB, which will be marketed as (b) (4), is a fixed-dose combination of GLE, an investigational HCV nonstructural (NS) 3/4A protease inhibitor and PIB, an investigational HCV NS5A inhibitor. GLE and PIB each have demonstrated antiviral activity against genotype (GT) 1 through 6 in cell culture, with more potency against common NS3/4A and NS5A resistance-associated substitutions in cell culture based phenotypic assays. The sponsor has concluded that GLE/PIB is indicated for the treatment of HCV GT 1, 2, 3, 4, 5, and 6 infection, including treatment-naïve and -experienced patients, and patients with compensated cirrhosis, renal failure, and coinfection with human immunodeficiency virus. This review focused on the independent assessment of next generation sequencing (NGS) data provided in support of the NDA. Overall, assessment of the NGS data by the Division of Antiviral Products (DAVP) indicated that the data and analyses provided by the sponsor, AbbVie, Inc., were acceptable and this NDA is approvable with respect to the NGS analyses performed by Clinical Virology.

GLE is an inhibitor of the HCV NS3/4A protease, which is essential for proteolytically processing the HCV polyprotein, and required for viral replication and assembly. GLE was evaluated in both HCV NS3/4A protease cleavage enzyme assays and HCV subgenomic replicon cell-based assays for inhibition of HCV. In enzymatic assays, GLE inhibited NS3 protease enzymes from HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a with IC_{50} values of 4.6, 8.9, 3.5, 3.8, 7.9, 6.1, 8.1 and 11.3 nM, respectively. GLE inhibited replication of HCV subgenomic replicons in stable cell lines with EC_{50} values of 0.85, 0.94, 2.2, 4.6, 1.9, 2.8 and 0.86 nM against genotypes 1a-H77, 1b-Con1, 2a-JFH1 and chimeric replicons containing NS3 from genotype 2b, 3a, 4a and 6a clinical samples, respectively. GLE exhibited activity against a panel of 40 clinical samples from HCV GT 1a, 1b, 2a, 2b, 3a, 4a, 4d and 5a infected subjects with a median EC_{50} value of 0.30 nM (range 0.05 to 3.8 nM) in the cell culture based replicon transient transfection assay. The most common resistance-associated substitutions (RAS) identified in cell culture were at positions 156 and 168.

PIB is an inhibitor of the HCV NS5A protein, which is required for viral replication and assembly. Against HCV GT 1a-H77, 1b-Con1 and chimeric replicons containing NS5A from GT 2a, 2b, 3a, 4a, 5a and 6a clinical samples, PIB inhibited replication of HCV subgenomic replicons in stable cell lines with EC_{50} values of 1.8, 4.3, 2.3, 1.9, 2.1, 1.9, 1.4 and 2.8 pM, respectively. In addition, PIB exhibited antiviral activity against a panel of 74 clinical samples from HCV GT 1-6 infected subjects, with a median EC_{50} value of 1.1 pM (range 0.27 to 3.5 pM) in the cell culture replicon transient transfection assay. The most common cell culture-based RAS for PIB was Y93H/N; however, the combinations of F28S + M31I or P29S + K30G or A30K + Y93H were also associated with resistance to PIB.

The clinical development program for the combination treatment regimen of once-daily (QD) GLE/PIB (300/120 mg; administered as co-formulated tablets) included efficacy and safety data from 8 registrational clinical studies, including clinical trials M15-464, M13-594, M13-590, M13-583, M14-172, M15-462, M14-868 Parts 3 and 4, and M15-410 Part 2, and 3 supportive Phase 2 studies M14-867 Part 2, M14-868 Parts 1 and 2, and M15-410 Part 1. The sponsor reported that a sustained virologic response 12 weeks post-treatment (SVR12) was achieved in 86.4% – 100% of subjects across the 21 treatment arms administered the GLE/PIB (300/120 mg). Please see the review of Senior Clinical Virology Reviewer, Dr. Patrick Harrington, Ph.D. for specific information (NDA 209394 SDN 000).

Given that there were more treatment failures among subjects who received prior treatment with a direct-acting antiviral (DAA) against HCV, the NGS analyses for this NDA was focused on sequences

obtained for subjects who failed treatment in MAGELLAN-1 (M15-410). In this study, the sponsor assessed the efficacy and safety of GLE/PIB (300/120 mg) in subjects who had received prior DAAs, including protease inhibitors, NS5A inhibitors, and nucleotide analog NS5B polymerase inhibitors. In M15-410 Part 1, 22 HCV GT1-infected, DAA treatment-experienced (TE) subjects without cirrhosis were treated for 12 weeks and 19 subjects in this group achieved SVR12 for a rate of 86.4% (95% CI: 66.7, 95.3). In Part 2, Arm D, 44 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 12 weeks and 39 subjects in this group achieved SVR12 for a rate of 88.6% (95% CI: 76.0, 95.0). In Part 2, Arm E, 47 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 16 weeks and 43 subjects in this group achieved SVR12 for a rate of 91.5% (95% CI: 80.1, 96.6)(see NDA 209394 SDN 000 statistical review for more details).

For MAGELLAN-1 (M15-410) Part 1, Arm C, 1 subject experienced virologic breakthrough on treatment and 2 subjects were described as non-virologic failures due to early discontinuation. In Part 2, Arm D there were 5 treatment failures, with 1 subject experiencing virologic breakthrough and 4 subjects who experienced relapse. In Part 2, Arm E there were 4 treatment failures, with all 4 subjects experiencing virologic breakthrough on treatment. The NGS resistance analysis performed for this review focused on these 11 subjects.

NGS data were generated by sequencing serum samples collected at baseline for all subjects and at a time point near the time of treatment failure for subjects who failed the GLE/PIB treatment to assess for amino acid substitutions in the viral target protein that could confer resistance, detected as amino acid substitutions that correlate with treatment failure, to one of the drugs in the GLE/PIB FDC. Several amino acid positions have been associated with resistance to GLE and PIB. For GLE, substitutions at NS3 positions 36, 54, 55, 56, 80, 122, 132, 155, 168, and 170 (see NDA 209394 SDN 000) are considered to be resistance-associated. For PIB, the following NS5A positions were determined to be resistance-associated: 24, 28, 30, 31, 32, 58, 92, and 93 (see NDA 209394 SDN 000).

The goal of the independent assessment of NGS data was to confirm the results reported by the sponsor, to determine which known resistance-associated substitutions were present in the virus of subjects who failed treatment, and to determine if additional substitutions occurring in two or more subjects could be associated with treatment failure.

Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few minor exceptions. No additional substitutions that occurred in two or more subjects could be determined to be associated with resistance for the subjects analyzed.

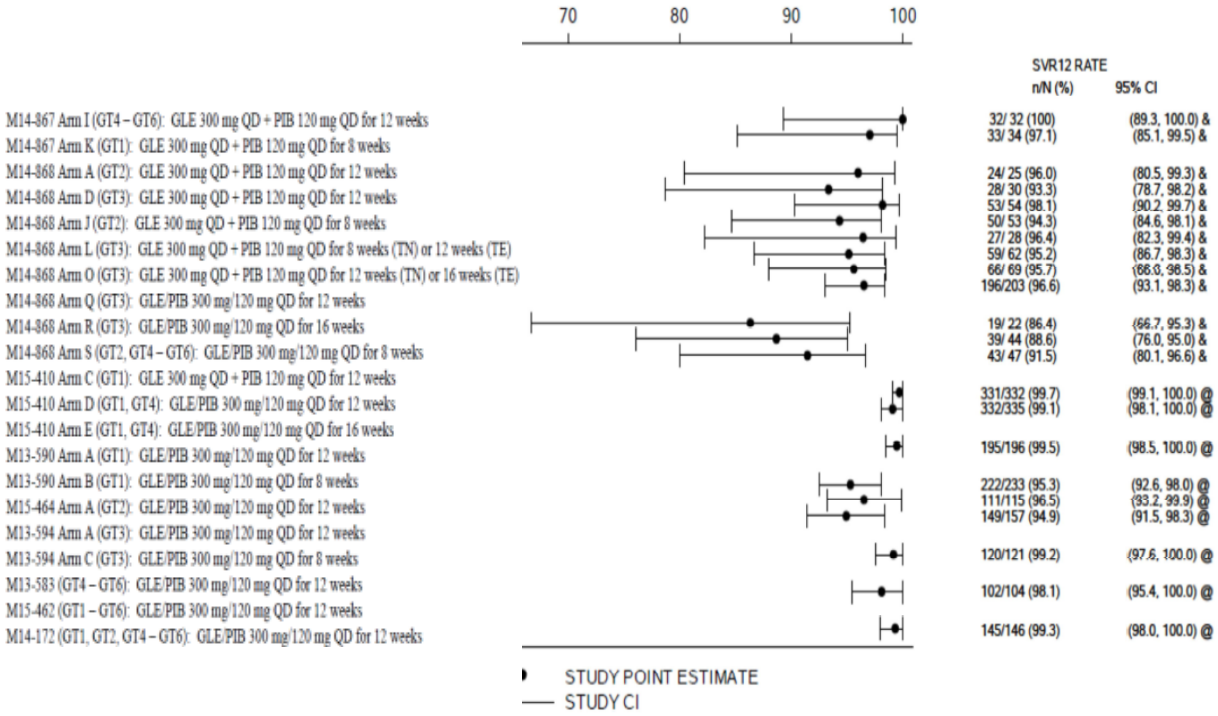
BACKGROUND AND SUMMARY

GLE is an inhibitor of the HCV NS3/4A protease, which is essential for proteolytically processing the HCV polyprotein into mature proteins (NS3, NS4A, NS4B, NS5A and NS5B) and is required for viral replication and assembly. GLE was evaluated in both HCV NS3/4A protease cleavage enzyme assays and HCV subgenomic replicon cell-based assays for inhibition of HCV. In enzymatic assays using purified NS3 protease, GLE inhibited purified NS3 protease enzymes from HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a with IC_{50} values of 4.6, 8.9, 3.5, 3.8, 7.9, 6.1, 8.1 and 11.3 nM, respectively. GLE inhibited replication of HCV subgenomic replicons in stable cell lines with EC_{50} values of 0.85, 0.94, 2.2, 4.6, 1.9, 2.8 and 0.86 nM against genotypes 1a-H77, 1b-Con1, 2a-JFH1 and chimeric replicons containing NS3 from genotype 2b, 3a, 4a and 6a clinical samples, respectively. GLE retained its activity against a panel of 40 clinical samples from HCV GT 1a, 1b, 2a, 2b, 3a, 4a, 4d and 5a infected subjects, with a median EC_{50} value of 0.30 nM (range 0.05 to 3.8 nM) in the cell culture based replicon transient transfection assay. The most common cell culture-based RAS were at positions 156 and 168.

PIB is an inhibitor of the HCV NS5A protein, which is required for viral replication and assembly, although it's precise role in those processes has not yet been fully characterized. Against HCV GT 1a-H77, 1b-Con1 and chimeric replicons containing NS5A from GT 2a, 2b, 3a, 4a, 5a and 6a clinical samples, PIB inhibited replication of HCV subgenomic replicons in stable cell lines with EC_{50} values of

1.8, 4.3, 2.3, 1.9, 2.1, 1.9, 1.4 and 2.8 pM, respectively. In addition, PIB exhibited antiviral activity against a panel of 74 clinical samples from HCV GT 1-6 infected subjects, with a median EC_{50} value of 1.1 pM (range 0.27 to 3.5 pM) in the cell culture replicon transient transfection assay. The most common cell culture-based RAS for PIB was Y93H/N; however, the combinations of F28S + M31I or P29S + K30G or A30K + Y93H were also associated with resistance to PIB.

The clinical development program for the combination treatment regimen of once-daily (QD) GLE/PIB (300/120 mg; administered as co-formulated tablets) included efficacy and safety data from 8 clinical studies, including clinical trials M15-464, M13-594, M13-590, M13-583, M14-172, M15-462, M14-868 Parts 3 and 4, and M15-410 Part 2, and 3 supportive Phase 2 studies M14-867 Part 2, M14-868 Parts 1 and 2, and M15-410 Part 1. According to the sponsor SVR12 was achieved in 86.4% – 100% of subjects across the 21 treatment arms administered the GLE/PIB (300/120 mg) QD, according to the protocol-specified primary endpoint population and confidence interval (Figure 1).



CI = confidence interval; DCV = daclatasvir; GLE = glecaprevir; GT=genotype; ITT = intention-to-treat; PIB = pibrentasvir; PS = primary subset; SVR_{12} = sustained virologic response 12 weeks postdosing; QD = once daily; RBV = ribavirin; SOF = sofosbuvir; SOF/R = regimens containing SOF and ribavirin; TE = treatment-experienced; TN = treatment-naïve

@ CI = CI calculated using the normal approximation to the binomial distribution.

& CI = CI calculated using the Wilson's score method.

Notes: ITT population used for all treatment arms except that Study M15-464 Arm A excludes SOF/R-experienced subjects and Study M13-590 Arms A and B are in the ITT-PS population. Arm B (SOF 400 mg QD + DCV 60 mg QD for 12 weeks) of Study M13-594 is not included in the Phase 2 and 3 Analysis Set but included in this forest plot.

Figure 1. Forest plot of SVR12 rates and 95% CIs of all treatment arms (Phase 2 and 3 Analysis Set) (Figure 1, page 50, Clinical Efficacy for HCV Summary).

For MAGELLAN-1 (M15-410), the sponsor assessed the efficacy and safety of GLE/PIB (300/120 mg) in subjects who had received prior DAAs, including protease inhibitors, NS5A inhibitors, and nucleotide analog NS5B polymerase inhibitors. In M15-410 Part 1, 22 HCV GT1-infected, DAA treatment-experienced (TE) subjects without cirrhosis were treated for 12 weeks and 19 subjects in this group achieved SVR12 for a rate of 86.4% (95% CI: 66.7, 95.3). In Part 2, Arm D, 44 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 12 weeks and 39 subjects in this group achieved SVR12 for a rate of 88.6% (95% CI: 76.0, 95.0). In Part 2, Arm E, 47 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 16 weeks and 43 subjects in this

group achieved SVR12 for a rate of 91.5% (95% CI: 80.1, 96.6)(see NDA 209394 SDN 000 statistical review for more details) (Table 1).

Table 1. Results of Efficacy Studies – MAGELLAN-1 (M15-410) (Table 4, page 28, Clinical Efficacy for HCV Summary).

Study ID (Genotypes)	Treatment Arm	No. Treated/ Completed	SVR_{12} Results n/N (%), 95% CI[a]	Hypothesis Test Results
M15-410 Part 1 (GT1)	Part 1 Arm C (HCV GT1-infected, DAA TE subjects without cirrhosis): GLE 300 mg QD + PIB 120 mg QD for 12 weeks	Part 1 Arm C: 22/21	Part 1 Arm C: 19/22 (86.4%); 66.7, 95.3	Not applicable
M15-410 Part 2 (GT1 and GT4 – GT6)	Part 2 (HCV GT1- or GT4- – GT6-infected, DAA TE subjects with or without cirrhosis): Arm D: GLE/PIB 300 mg/120 mg QD for 12 weeks Arm E: GLE/PIB 300 mg/120 mg QD for 16 weeks	Part 2 Arm D: 44/44 Part 2 Arm E: 47/43	Part 2 Arm D: 39/44 (88.6%), 76.0, 95.0 Part 2 Arm E: 43/47 (91.5%), 80.1, 96.6	Not applicable

CI = confidence interval; DAA = direct-acting antiviral agent; DCV = daclatasvir; DSV = dasabuvir; GLE = glecaprevir; GT = genotype; HCV = hepatitis C virus; LCB = lower confidence bound; PIB = pibrentasvir; pegIFN = pegylated interferon; PP = per protocol; ITT = intention-to-treat; ITT-PS = intention-to-treat subset of monoinfected HCV GT1 DAA-naïve subjects; OBV = ombitasvir; PTV = paritaprevir; QD = once daily; RBV = ribavirin; SOF = sofosbuvir; SOF/R = regimens containing SOF and RBV; SVR_{12} = sustained virologic response 12 weeks postdosing; TE = treatment-experienced; TN = treatment-naïve

a. The CI calculations used the protocol-specified method. Additional CIs for SVR_{12} rate for each arm were calculated using Wilson's score methods in ISE (R&D/16/0156) Table 1.2__1.1.

b. Two subjects assigned to Arm I who took GLE 200 mg QD + PIB 120 mg QD for 12 weeks are counted as treated in Arm A in the ISE tables.

The number and percentage of subjects with on-treatment virologic failure and with relapse before or during the SVR12 window by treatment arm in the MAGELLAN-1 (M15-410) study are presented in Table 2.

Table 2. Responses for subjects in MAGELLAN-1 (M15-410) Arms C, D, and E (Table 1, page 28, RD160492 Resistance Report).

Study	Study Design	Duration	Total Enrolled	Number with of Subjects Baseline Sequence[a]		
				PVF	Non-PVF	SVR12
M14-867 (Part 2 – Arms I, K)	An Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of GLE and PIB With and Without Ribavirin in Subjects with Chronic HCV GT 1, 4, 5, and 6 Infection (SURVEYOR-I)	8 and 12 weeks	68	0	1	65
M14-868 (Arms A, D, J, L, O, Q, R and S)	A Randomized, Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of GLE and PIB With and Without RBV in Subjects With Chronic HCV GT 2, 3, 4, 5 or 6 Infection (SURVEYOR-II)	8, 12, and 16 weeks	524	11	9	478
M15-410 (Arms C, D, E)	A Randomized, Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of GLE and PIB (or ABT-493/ABT-530) With and Without Ribavirin in Adults With Chronic HCV Infection who Failed a Prior Direct-Acting Antiviral Agent-Containing Therapy	12 and 16 weeks	113	10	2	99

GLE = Glecaprevir, ABT-493; PIB = pibrentasvir, ABT-530; GT = genotype; HCV = hepatitis C virus; PVF = primary virologic failure population (includes on-treatment virologic failure and post-treatment relapse, and does not include subjects not achieving SVR_{12} due to non-virologic reasons); non-PVF = subjects not achieving SVR_{12} due to non-virologic reasons such as premature study drug discontinuations, missing SVR_{12} data, etc.

a. Indicates total number of subjects for whom baseline NS3/4A and/or NS5A sequences were available. Number of sequences available for each target may differ.

Given that there were more treatment failures among subjects who received prior treatment with a direct-acting antiviral (DAA) against HCV, the NGS analyses for this NDA was focused on sequences obtained for subjects who failed treatment in MAGELLAN-1 (M15-410). Next generation sequencing of

the HCV NS3/4A, and NS5A genes was performed for all subjects at baseline and at the timepoint closest to failure for all subjects who relapsed or otherwise met the criteria for treatment failure in 9 phase 2 and 3 clinical trials.

The sponsor provided 5,216 fastq files as part of their resistance analysis for the 8 clinical trials (see Figure 1 for the list of clinical trials). The sponsor included an Integrated Resistance Analysis report, which contained the integrated analyses conducted for the 8 clinical studies evaluating GLE/PIB (300/120 mg) in subjects with chronic HCV infection with genotypes 1 through 6 with or without cirrhosis. The NGS data provided by the sponsor included: 1) frequency tables showing amino acid variation that occurred at each position of the three viral proteins (NS3/4A and NS5A) for each subject at baseline and near the time of failure for all treatment failure samples that were successfully sequenced using Illumina technology; 2) raw sequence data in fastq format for all samples that were deep sequenced; 3) summary resistance data for each study; and 4) cross study comparisons of resistance data.

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 DAVP 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 an optimized NGS analysis pipeline in the High-Performance Integrative Virtual Environment (HIVE). A second optimized NGS analysis pipeline using CLC Genomics Workbench was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency) between the sponsor's results and the HIVE pipeline. The results of the independent assessment 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 when applicable.

Rationale for Requesting and Analyzing NGS Data

In general, the FDA does not analyze raw nucleotide sequence data, only derived data for Sanger sequencing, in conjunction with new drug applications (NDAs). However, because of the complexity and size of NGS data, the lack of data standards and standardized analyses pathways, and the large number of tools available for different operations in an analysis pathway the Division of Antiviral Products has determined that an independent analysis of NGS data should be performed for new drug applications to ensure that resistance pathways for new drugs and new combinations are carefully characterized and defined in the label. DAVP provides an NGS template entitled, ***Submitting Next Generation Sequencing Data to the Division of Antiviral Products*** to sponsors who intend to use NGS technology in support of new drug approvals.

NGS Data Analysis Pipeline

DAVP used the High-Performance Integrated Virtual Environment (HIVE) (Simonyan et al, 2016), in collaboration with CBER, to perform the majority of the independent assessment of the NGS data for this NDA. DAVP virologists, in collaboration with the HIVE team, have developed specific tools that allow DAVP to batch rename files to meet nomenclature rules for the analysis pipeline, to assess quality control on all sequence files, to convert variant call files (VCF) to frequency tables at the amino acid level, and to generate tables comparing results submitted by the sponsor and those generated by HIVE or CLC Genomics (Figure 2). The initial analysis of NGS data was generated using HIVE. A

second optimized NGS analysis pipeline using CLC Genomics Workbench was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency of 10% or more) between the sponsor's results and the HIVE pipeline (Figure 2).

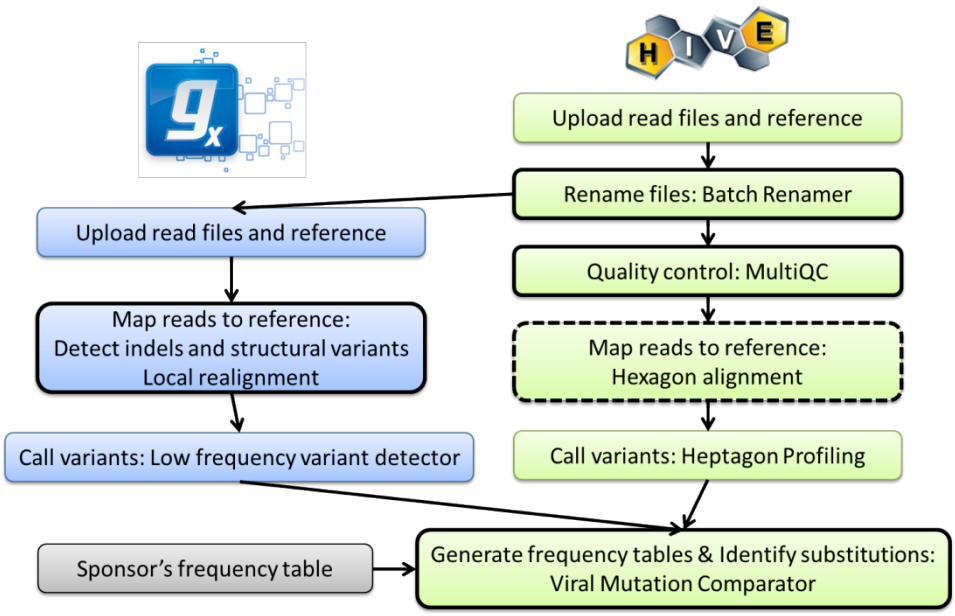


Figure 2. Overview of the NGS analysis pipeline using HIVE and/or CLC Genomics Workbench (DAVP Analysis).

NGS Analysis Parameters and Overview of Data Analysis

DAVP used an optimized HIVE analysis pipeline for the primary NGS analyses and an optimized CLC Genomics workbench pipeline for the secondary NGS analyses (assessing disagreements). The workflow in HIVE was as follows: 1) sequence reads were mapped to the reference sequence using Hexagon aligner tool, 2) variants were called at the amino acid level using the Heptagon profiling tool, and 3) frequency tables were generated using the Viral Mutation Comparator tool.

The parameters used in HIVE were the following:

1. Mapping using Hexagon aligner
    a. Match benefit: 5
    b. Mismatch penalty: -4
    c. Mismatch continuation penalty: -6
    d. Gap continuation cost: -4
    e. Gap opening cost: -12
    f. Local alignment
2. Heptagon profiling
    a. Minimum coverage was set to 100

Briefly, the workflow in CLC Genomics workbench was as follows: 1) trimmed reads were mapped to the reference sequence, 2) structural variants were detected based on the information derived from unaligned ends and locally realigned to the reference sequence, 3) consensus sequences were generated, 4) variants were called at the amino acid level, exported in Excel, and later imported into HIVE for analysis using HIVE's Viral Mutation Comparator tool and to generate frequency tables.
The parameters used in CLC Genomics were the following:

1. Mapping using Hexagon aligner
    a. Match score: 1

   b. Mismatch cost: 2
   c. Insertion cost: 3
   d. Deletion cost: 3
   e. Length fraction of read required for mapping: 0.5
   f. Similarity fraction: 0.8
   g. Local alignment
2. InDels and Structural Variants
   a. p-value threshold of unaligned end breakpoints: 0.0001
   b. maximum number of mismatches: 3
   c. minimum number of reads to filter variants: 2
3. Local realignment
   a. multi-pass realignment: 2
   b. maximum guidance-variant length (defined by InDels and Structural Variants): 100
4. Low frequency variant detection
   a. Minimum frequency: 1%
   b. Minimum coverage was set to 100
   c. Frequency was set to 1%
   d. Significance: 1%

Each step of the analysis process is briefly described below.

1. **Processing fastq files**. Data were submitted to the FDA on a portable hard drive, which included fastq files for each subject (at baseline and a timepoint close to the time of failure) that was sequenced using paired end chemistry and the Illumina sequencing platform. The sequences were uploaded via the HIVE interface and assessed for quality control by looking at the following parameters: position statistics, where the mean phred score is calculated; read length, the relative base population of A, C, G, T for each sample; and average base quality for each file. Outlier files were flagged and evaluated more closely.
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 and NS5A genes for HCV GT1a (H77; NC_004102), GT1b (Con1; AJ238799), GT1c (HC-G9; D14853), GT1e (QC172; KJ439769), and GT4r (QC384; FJ462439) were imported and annotated as coding sequences to be used as reference sequences for mapping. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench (when used).
3. **Mapping reads to the appropriate reference sequence for each HCV genotype/subtype.** The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each sample. The mapping contained the target of interest (the NS3/4A or NS5A gene sequences) and was used to identify variants that differed from the reference sequence. 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.

NGS Analysis Pipeline Output

4. **Generating frequency tables of amino acid substitutions.** From the read mappings, variants were called and variant tables were generated for each sequence run using the built in variant caller in HIVE (Simonyan et al., 2017) and the low frequency variant caller in CLC Genomics workbench (only used in cases of disagreement between HIVE and the sponsor). The variant call tables were converted into amino acid frequency tables using the HIVE Viral Mutation Comparator tool. Below are descriptions for the variant callers and the frequency table:

a. **HIVE Variant Detector (HVD)** – calls variants from a read mapping using the Heptagon Sequence Profiler tool (Simonyan et al., 2017). Following alignment, the reference-based variant profile is computed by mapping nucleotides of short read sequences and counting occurrences of distinct bases at every genomic position on the reference. This variant-calling procedure produces consensus, per-base, forward/reverse, and total coverage maps for all reference segments of the specified reference genomes/sets. The frequencies of each variant call are then computed relative to the reference genome or relative to the accumulated consensus genome, depending on user specifications. Amino acids (AA) are called based on the contribution of every read and its alignment in relation to an annotated open reading frame (ORF). AA substitution calls are made from codon translations of individual reads, based on mapping to annotated open reading frames. This is to contrast the variant calling from a consensus based AA calling procedure.

b. **Low Frequency Variant Detection (LFVD)** – according to the CLC Genomics Workbench Manual: *"a statistical test is performed at each site to determine if the nucleotides observed in the reads at that site could be due simply to sequencing errors, or if they are significantly better explained by there being one (or more) alleles than the reference present in the sample at some unknown frequency. If the latter is the case, a variant corresponding to the significant allele will be called, with estimated frequency".*

c. **Frequency tables** – Tables were generated using the HIVE Viral Mutation Comparator tool. This table contains information for each position of each gene and each subject for which variation from the reference occurs. The frequency table contains the following columns: unique subject identifier (USUBJID), treatment regimen (ARM), visit (VISIT), the amino acid position within the gene of interest (AAPOS), total coverage at the nucleotide position (TCOV), the amino acid found in the reference sequence (AAREF), the amino acid substitution (AASUB), the coverage at the nucleotide level for the variant (VCOV), and the frequency by which the variant was detected (AAFREQ). Frequency tables are generated by:

   i. The variant tables were combined by genotype/subtype and study

   ii. The variant tables were filtered to remove synonymous substitutions

   iii. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the sponsor

   iv. Any amino acid substitution ≥1%

5. **Generating resistance analysis tables.** The HIVE Viral Comparator tool and Excel macros were used 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.

6. **Conducting independent resistance analysis.** The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above a defined frequency threshold of 10%, using the following criteria:

   a. **SUBS10 criteria** – Identified all substitutions that were not detected at baseline (<0.01 frequency) but were detected at a frequency of 10% (0.10) or greater at later timepoints or detected at baseline at a frequency of 10% (0.10) and not detected at later timepoints.

b. In addition, known resistance-associated substitutions that have been identified in NS3/4A and NS5A were analyzed at the 1% frequency cutoff, given that amino acid replacements at these sites may represent viral populations that are of low fitness and therefore may rapidly be overgrown once treatment stops. Several amino acid positions have been associated with resistance to GLE and PIB. For GLE, substitutions at NS3 positions V36, T54, V55, Y56, Q80, S122, V132, R155, A156, D168, and V170 are considered to be resistance-associated. For PIB, the following NS5A positions were determined to be resistance-associated: K24, M28, Q30, L31, P32, H58, A92, and Y93.

NGS Data Comparison

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 were 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 at least two and up to three algorithms, including the sponsor's algorithm (ABV) and HVD and LFVD (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 when applicable
    e. Novel resistance-associated substitutions identified by the independent analysis were noted and discussed with the review team for potential labeling/post-marketing actions

CLINICAL STUDIES

The clinical development program for the combination treatment regimen of once-daily (QD) GLE/PIB (300/120 mg; administered as co-formulated tablets) included efficacy and safety data from 8 clinical studies, including clinical trials M15-464, M13-594, M13-590, M13-583, M14-172, M15-462, M14-868 Parts 3 and 4, and M15-410 Part 2, and 3 supportive Phase 2 studies M14-867 Part 2, M14-868 Parts 1 and 2, and M15-410 Part 1.

The sponsor provided 5,216 fastq files as part of their resistance analysis for the 8 clinical trials (see Figure 1 for the list of clinical trials). The sponsor included an Integrated Resistance Analysis report, which contained the integrated analyses conducted for the 8 clinical studies evaluating GLE/PIB (300/120 mg) in subjects with chronic HCV infection with genotypes 1 through 6 with or without cirrhosis. The NGS data provided by the sponsor included: 1) frequency tables showing amino acid variation that occurred at each position of the three viral proteins (NS3/4A and NS5A) for each subject at baseline and near the time of failure for all treatment failure samples that were successfully sequenced using Illumina technology; 2) raw sequence data in fastq format for all samples that were deep sequenced; 3) summary resistance data for each study; and 4) cross study comparisons of resistance data.

Given that there were more virologic failures among subjects who received prior treatment with a direct-acting antiviral (DAA) against HCV and few virologic failures among the treatment-naïve subjects, the NGS analyses for this NDA was focused on sequences obtained for subjects who failed treatment in MAGELLAN-1 (M15-410). Next generation sequencing of the HCV NS3/4A, and NS5A genes was

performed for all subjects at baseline and at the timepoint closest to failure for all subjects who relapsed or otherwise met the criteria for treatment failure in the 9 phase 2 and 3 clinical trials. This portion of the clinical virology NDA review focused exclusively on the independent analysis of NGS data from treatment failures from the phase 2 clinical trial MAGELLAN-1 (M15-410, Part 2). The primary review of the phase 3 and phase 2 clinical trials and overall conclusions drawn from phase 2 and phase 3 resistance data can be found in the review of Senior Clinical Virology Reviewer Patrick Harrington, Ph.D. (NDA 209394 SDN 000).

For MAGELLAN-1 (M15-410), the sponsor assessed the efficacy and safety of GLE/PIB (300/120 mg) in subjects who had received prior DAAs, including protease inhibitors, NS5A inhibitors, and nucleoside analog NS5B polymerase inhibitors. In M15-410 Part 1, 22 HCV GT1-infected, DAA treatment-experienced (TE) subjects without cirrhosis were treated for 12 weeks and 19 subjects in this group achieved SVR12 for a rate of 86.4% (95% CI: 66.7, 95.3). In Part 2, Arm D, 44 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 12 weeks and 39 subjects in this group achieved SVR12 for a rate of 88.6% (95% CI: 76.0, 95.0). In Part 2, Arm E, 47 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 16 weeks and 43 subjects in this group achieved SVR12 for a rate of 91.5% (95% CI: 80.1, 96.6)(see NDA 209394 SDN 000 statistical review for more details). There were a total of 306 fastq sequences submitted for Part 2 of this study, which included baseline data for 128 subjects who achieved SVR12 (GT1a=97, GT1b=25, GT1c=1, GT1e=1, GT4a/c/d=1, and GT4r=1). NGS data that included sequences taken at baseline and at least one post-treatment timepoint were provided for 11 subjects who were consider treatment failures (6 subjects experienced virologic breakthrough (GT1a=6) and 5 subjects relapsed (GT1a=4, GT1b=1) and two subjects designated non-virologic failure discontinuations (both were infected with GT1a) (Table 3).

Table 3. MAGELLAN-1 (M15-410) subjects analyzed by NGS (DAVP analysis).

	GT1a	GT1b	GT1c	GT1e	GT4a/c/d	GT4r	Totals	Fastq files	Issues
No. SVR	97	25	1	1	1	3	**128**	256	Corrupt file for subject 2125, missing files for GT1c and GT4a/c/d subjects
On TX VF	6	0	0	0	0	0	**6**	24	No issues
Relapse	4	1	0	0	0	0	**5**	22	No issues
Non-VF	2	0	0	0	0	0	**2**	4	No issues
Total	**109**	**26**	**1**	**1**	**1**	**3**	141	**306**	Totals are correct

N=2 files per sample, but in some cases samples were sequenced more than once (Relapse)

There was one corrupt file and missing data for three subjects, all of whom achieved SVR, and these were determined to be nonessential for review of the NGS results

The sponsor submitted an Integrated Resistance Analysis Report that described the individual and integrated resistance analyses results for the 8 clinical studies that evaluated the GLE/PIB in treatment-naïve and treatment-experienced subjects chronically infected with HCV genotype 1 through 6. The results reported for MAGELLAN-1 (M15-410) were compared to the results generated independently by DAVP.

Resistance Analysis Population

As stated in the Integrated Resistance Analysis Report, the resistance analysis population for MAGELLAN-1 (M15-410) included any subject who received at least 1 dose of a regimen containing GLE/PIB (300/120 mg). The integrated resistance analyses only included data from subjects in arms administered ABT-493 300 mg QD and ABT-530 120 mg QD in Phase 2 or 3 studies. Baseline and post-baseline samples were sequenced for these subjects, following these guidelines:

1. **Baseline sequencing:** The integrated resistance analyses included available NGS data from available baseline samples for all subjects treated with ABT-493 300 mg QD and ABT-530 120 mg QD.

2. **Post-baseline sequencing:** The sponsor's criteria for integrated resistance analyses included subjects treated with study drug (ABT-493 300 mg QD and ABT-530 120 mg QD) who did not achieve SVR12 as per the following criteria: (1) if they had on-treatment breakthrough; (2) if they had post-treatment relapse, with a study drug duration ≥52 days, ≥77 days, and ≥105 days for 8-week, 12-week and 16-week treatment assignment, respectively; or (3) if they had HCV RNA ≥LLOQ at end of treatment with at least 6 weeks of treatment. Subjects meeting one of these criteria were referred to as subjects in the primary virologic failure (PVF) population. Subjects treated with study drug who did not achieve SVR12 and did not meet the above criteria for the PVF population (e.g., those with less than 6 weeks of therapy who have HCV RNA ≥LLOQ at end of treatment), but have a time point with HCV RNA ≥1,000 IU/mL after treatment discontinuation, had a sample at that time point sequenced. These subjects were referred to as subjects in the non-PVF population. For all subjects randomized to any treatment arm included in the primary analysis in the listed Phase 2 and 3 studies who were in the PVF or non-PVF population, available NGS data from the post-baseline time point (at failure or after treatment discontinuation) were included in all analyses of post-baseline substitutions.

The sponsor used the following definitions for Efficacy Endpoints that were pertinent to this review. Please note that additional Efficacy Endpoints were defined and used in the NDA (see the review of Patrick Harrington, Ph.D. for more details – NDA 209394 SDN 000). A confirmed quantifiable post-treatment value was defined as any 2 consecutive post-treatment HCV RNA measurements ≥LLOQ.

1. **Breakthrough** = confirmed HCV RNA ≥100 IU/mL after HCV RNA <LLOQ during the Treatment Period; or confirmed increase from nadir in HCV RNA (2 consecutive HCV RNA measurements >1 $\log_{10}$ IU/mL above nadir) at any time point during the Treatment Period. A single breakthrough value (≥100 IU/mL or >1 $\log_{10}$ above nadir) followed by lost to follow-up would also be considered a breakthrough (i.e., will not require confirmation).
2. **EOT failure** = HCV RNA ≥LLOQ at end of treatment with at least 6 weeks of treatment, where the HCV RNA value must be collected on or after Study Drug Day 36 and study drug duration ≥36 days.
3. **On-treatment virologic failure** = Breakthrough or EOT failure.
4. **Virologic failure** = On-treatment virologic failure or relapse.
5. **SVR12** = HCV RNA <LLOQ in the SVR12 window (12 weeks after the last actual dose of active study drug) without any confirmed quantifiable (≥LLOQ) post-treatment value before or during that SVR window.
6. **Relapse** = confirmed HCV RNA ≥LLOQ between end of treatment and up to and including the last HCV RNA measurement collected in the post-treatment Period for a subject with HCV RNA <LLOQ at Final Treatment Visit who completes treatment, excluding reinfection as described below.

Resistance Analysis Methodologies Used by the Sponsor

The sponsor stated that the HCV genotype and subtype were determined at screening by the central laboratory using Sanger sequencing or Inno-LiPA 2.0 Assay (if Sanger sequencing was not available) and verified by phylogenetic analysis. However, the sponsor noted that for HCV GT 2, 4, and 6 the LiPA 2.0 assay is unable to accurately identify the viral subtype. Therefore, for instances where a viral subtype could not be determined by LiPA 2.0, the viral subtype was determined by phylogenetic analysis of a 329 nucleotide region of NS5B that was RT-PCR amplified from the baseline samples (with HCV RNA ≥1,000 IU/mL) of HCV-infected subjects.

The sponsor reported that NGS analysis of HCV NS3/4A and NS5A coding regions was performed by [redacted] (b) (4) using reverse transcriptase polymerase

chain reaction (RT-PCR) and then followed by NGS analysis using the Illumina MiSeq deep sequencing platform (see the methods section in Appendix I).

Briefly, PCR amplicons supplied to (b) (4) by AbbVie were purified using Ampure XP beads (Beckman Coulter Genomics), and quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies). The DNA was then fragmented and tagged using the Nextera XT sample preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Index primers were added by limited cycle PCR using the Nextera XT Index kit (Illumina, San Diego, CA), and samples were normalized using beads with maximum binding capacity according to the Nextera XT sample preparation kit instructions. Multiplexed paired-end sequencing was conducted on the Illumina MiSeq platform using the MiSeq v2 sequencing kit with 300 cycles (Illumina). De-multiplexed fastq files were then mapped against the reference sequence using CLC Genomics Workbench software (CLCBio, Denmark). Sequences were trimmed to remove nucleotides with a quality score lower than 30. The attempted minimum coverage was 5,000 sequencing reads per nucleotide position. An amino acid variant report relative to a subtype-specific reference sequence was generated with the Athena pipeline proprietary software. The threshold for detection of nucleotide polymorphisms by NGS was set at 1% (The sponsor also used Monogram to perform NGS analyses and obtained similar results).

Although the threshold for detection of nucleotide polymorphisms by NGS was set at 1% by the sequencing vendors, the sponsor reported that they observed an unexpectedly high frequency of stop codons and polymorphisms at a number of positions across all sequences and subtypes, indicating a potential artifact within the sequence data. Therefore, the sponsor did an analysis of all baseline sequence data at 1% and 2% detection thresholds from Clinical Study M14-868 to establish a reliable lower bound of sensitivity. From this analysis, the sponsor concluded that a 2% cutoff for the lower bound of sensitivity was more appropriate for this dataset. During the independent analysis by DAVP, we also observed the high prevalence of stop codons detected just below 2%, and are in agreement with the sponsor's assessment. However, it is clear that some real resistance-associated substitutions (RAS) could be detected below 2% in some cases.

The sponsor performed baseline NGS analysis of the HCV NS3/4A and NS5A genes for all subjects in the mITT-VF population, which was defined as the ITT population excluding subjects who did not achieve SVR12 for reasons other than virologic reason. In addition, for all subjects who experienced virologic failure, NGS analysis was performed at the first time point after virologic failure if the plasma/serum sample was available and HCV RNA was >1,000 IU/mL.

The sponsor defined resistance-associated substitutions (RAS) and other variants as follows:

- **Baseline variant:** a variant by NGS in a baseline sample (≥2% or ≥15% prevalence within a subject's viral population depending on variant frequency threshold utilized) that was not present in the appropriate prototypic reference amino acid sequence for a given DAA target (NS3/4A or NS5A)
- **Variant at signature amino acid position:** variant (relative to reference) present in a baseline or a post-baseline sample at a signature amino acid position (Table 4)
- **Post-baseline variant:** an amino acid variant in a post-baseline time point sample that was not detected at baseline (<2%) in the subject and is detectable in ≥2% of the sequences from the post-baseline sample
- **Enriched variant:** variant present in both the baseline and a post-baseline sample whose prevalence in the post-baseline sample is at least 20 percentage points greater than the prevalence in the baseline sample [(post-baseline % – baseline %) ≥20]
- **Treatment-emergent variant by NGS:** A post-baseline variant or an enriched variant
- **Variants emerging at an amino acid position:** when 2 or more subjects of the same HCV subtype have treatment-emergent variants at an amino acid position (encompasses all variants at a given position)

- **Emerged variant:** a treatment-emergent variant that is observed in 2 or more subjects of the same HCV subtype (refers to a specific variant at a position)

The sponsor identified several amino acid positions of interest for NS3/4A protease inhibitors and for NS5A inhibitors and provided a list of positions for several genotypes (Table 4).

Table 4. List of genotype-specific signature amino acid positions and the key subset of amino acid positions across all genotypes (Table 3, page 32, RD160492 Resistance Report).

Genotype	Signature Amino Acid Positions	Key Subset of Amino Acid Positions
NS3/4A		
1a	36, 43, 54, 55, 56, 80, 107, 122, 132, 155, 156, 158, 168, 170	155, 156, 168 (all genotypes)
1b	36, 54, 55, 56, 80, 107, 122, 155, 156, 158, 168, 170, 175	
1 (other), 2, 3, 4, 5, 6	36, 43, 54, 55, 56, 80, 155, 156, 166 (GT3 only), 168	
NS5A		
1a	24, 28, 29, 30, 31, 32, 58, 62, 92, 93	24, 28, 30, 31, 58, 92, 93 (all genotypes)
1b	24, 28, 29, 30, 31, 32, 54, 58, 62, 92, 93	
1 (other), 2, 3, 4, 5, 6	24, 28, 29, 30, 31, 32, 58, 92, 93	

DAVP Assessment of MAGELLAN-1 (M15-410)

For MAGELLAN-1 (M15-410), the sponsor assessed the efficacy and safety of GLE/PIB (300/120 mg) in subjects who had received prior DAAs, including protease inhibitors, NS5A inhibitors, and nucleotide analog NS5B polymerase inhibitors. In M15-410 Part 1, 22 HCV GT1-infected, DAA treatment-experienced (TE) subjects without cirrhosis were treated for 12 weeks and 19 subjects in this group achieved SVR12 for a rate of 86.4% (95% CI: 66.7, 95.3). In Part 2, Arm D, 44 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 12 weeks and 39 subjects in this group achieved SVR12 for a rate of 88.6% (95% CI: 76.0, 95.0). In Part 2, Arm E, 47 HCV GT 1- or 4-infected, DAA TE subjects with or without cirrhosis were treated for 16 weeks and 43 subjects in this group achieved SVR12 for a rate of 91.5% (95% CI: 80.1, 96.6)(see NDA 209394 SDN 000 statistical review for more details). There were a total of 11 subjects from Part 2 of this study who did not achieve SVR12 because of virologic failure: 6 subjects experienced virologic breakthrough (GT1a=6) and 5 subjects relapsed (GT1a=4, GT1b=1). In addition, two subjects were designated non-virologic failures because they did not meet the mITT definition (both were infected with GT1a).

There were a total of 306 fastq sequences submitted for this Part 2 of this study, which included baseline data for 128 subjects who achieved SVR12 (GT1a=97, GT1b=25, GT1c=1, GT1e=1, GT4a/c/d=1, and GT4r=1). NGS data that included sequences taken at baseline and at least one post-treatment timepoint were provided for the 11 subjects who failed treatment with GLE/PIB. The two non-virologic failure subjects were not analyzed. The 306 fastq files were independently assessed and analyzed using the NGS analysis pipeline described above and the results were compared to those reported by the sponsor.

MAGELLAN-1 (M15-410) Baseline Analyses

A baseline analysis was performed by comparing amino acid substitutions (different from the reference sequence) detected in the HCV of subjects who failed treatment (n=11) to the amino acid substitutions detected in the HCV of subjects who achieved SVR12 (n=122) to determine if any baseline amino acid substitution could be associated with treatment failure (detected in 2 or more subjects who failed treatment but absent in subjects who achieved SVR12). Given that only one subject who failed was infected with HCV GT1b, the baseline analysis was only performed for subjects infected with HCV

GT1a who failed treatment (n=10) versus those achieving SVR12 (n=97). There were 28 amino acid positions in NS3/4A for which baseline amino acid substitutions were observed (Figure 3).

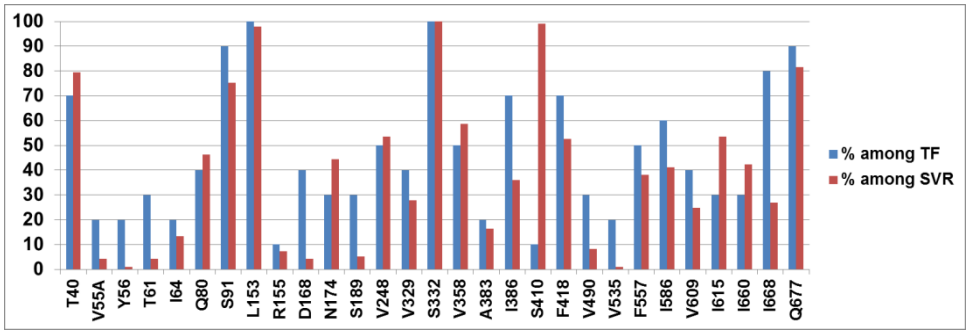


Figure 3. Baseline amino acid substitutions in NS3/4A compared between subjects infected with HCV GT1a who were treated with GLE/PIB and achieved SVR12 and those who failed treatment (DAVP Analysis). There were 10 subjects who failed treatment and 97 subjects who achieved SVR12.

In addition to baseline substitutions at NS3/4A positions Y56 and D168, which are known to be associated with protease inhibitor resistance, NS3/4A baseline substitutions were predominant among treatment failures at positions T61(n=2), S189 (n=3), V490 (n=3), V535 (n=2), and I668 (n=8). These additional sites are potentially resistance-associated, particularly position V535, which is highly conserved (no substitutions detected at baseline in any subjects who achieved SVR12). Of note, for the one subject infected with HCV GT1b who failed, none of the baseline substitutions detected at 15% or greater matched any of the baseline positions detected among treatment failures infected with HCV GT1a.

A baseline analysis was also performed comparing NS5A baseline amino acids detected in the HCV of subjects who failed treatment (n=11) versus subjects who achieved SVR12 (n=122) to identify potential baseline amino acid substitutions associated with treatment failure. Given that only one subject who failed was infected with HCV GT1b, this baseline analysis was also limited to subjects infected with HCV GT1a who failed treatment (n=10) versus those achieving SVR12 (n=97). There were 10 amino acid positions in NS5A for which baseline amino acid substitutions were detected in 2 or more subjects at ≥15% frequency (Figure 4).

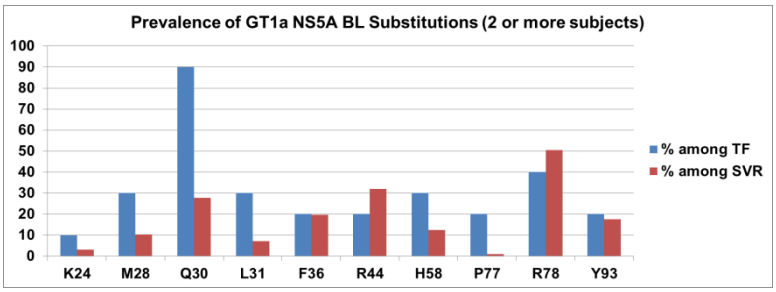


Figure 4. Baseline amino acid substitutions in NS5A compared between subjects infected with HCV GT1a who were treated with GLE/PIB and achieved SVR12 and those who failed treatment (DAVP Analysis). There were 10 subjects who failed treatment and 97 subjects who achieved SVR12.

Of the 10 baseline amino acids substitutions detected in NS5A, 6 occurred at amino acid positions that had already been associated with resistance to NS5A inhibitors: K24, M28, Q30, L31, H58, and Y93. Baseline amino acid substitutions at positions F36, R44, and R78 occurred at comparable frequency in the HCV of subjects who failed treatment and who achieved SVR12 indicating that these changes were likely to be polymorphic. A change at NS5A_P77 was predominantly in the NS5A of HCV GT1a in treatment failures; however, the sample size was small (n=2 in treatment failures, n=1 among subjects

who achieved SVR12). Of note, both treatment failures who had P77 at baseline in the NS5A gene of HCV GT1a had been treated with other NS5A inhibitors and also had other NS5A resistance-associated substitutions at baseline.

We next looked to see if there was a correlation between absolute concentration of a baseline resistance-associated substitution (frequency of amino acid substitution x baseline viral load) and treatment failure, based on an observation reported in the literature that in HIV-1 the absolute concentration of K103N at baseline predicted treatment success or failure for a particular antiretroviral therapy (Goodman et al., 2011). For this analysis, we focused on known RAS, which were defined as signature amino acid substitutions by the sponsor. In addition, since there was only one subject infected with HCV GT1b, we limited our analyses to the 10 subjects infected with HCV GT1a who failed treatment.

For NS3/4A, only RAS positions with baseline substitutions in the NS3/4A of the HCV GT1a for subjects who failed treatment and achieved SVR12 were compared (Figure 5). These positions included V36, T55, Y56, Q80, R155, and D168.

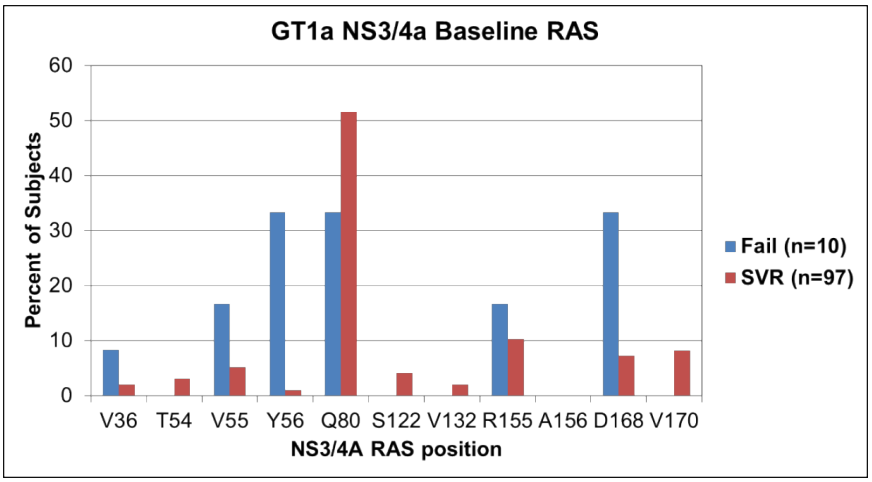


Figure 5. Baseline amino acid substitutions at RAS positions in NS3/4A compared between subjects infected with HCV GT1a who were treated with GLE/PIB and achieved SVR12 and those who failed treatment (DAVP Analysis). There were 10 subjects who failed treatment and 97 subjects who achieved SVR12.

The absolute concentration of V36 and Q80 at baseline was similar for both groups (data not shown); however, for positions T55 and Y56, the absolute baseline concentration was approximately 1 $\log_{10}$ higher in the virus of subjects who failed treatment, although the sample sizes were small (Figure 6).

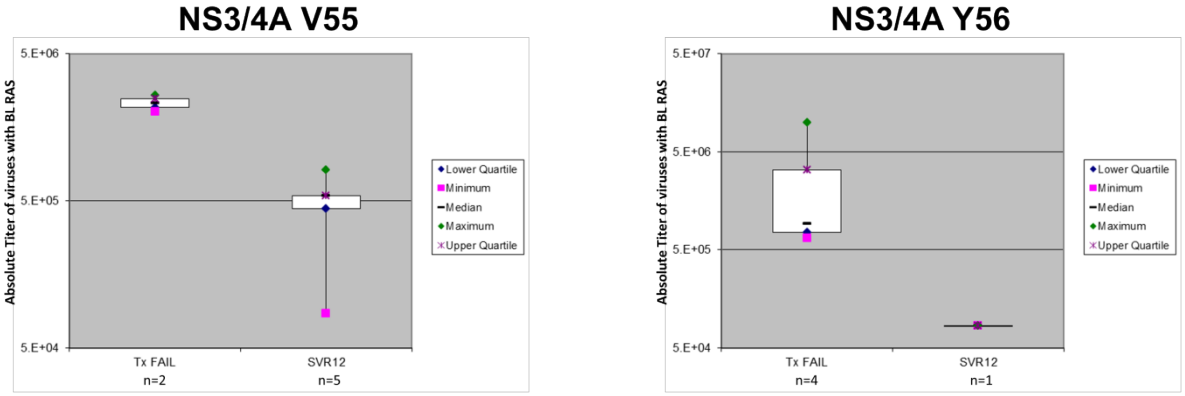


Figure 6. Absolute baseline concentration of NS3/4A V55 and Y56 RAS (DAVP analysis).

For positions R155 and D168, the absolute concentrations at baseline trended higher in the virus of subjects who failed treatment, but with overlapping ranges with those subjects who achieved SVR12 (Figure 7).

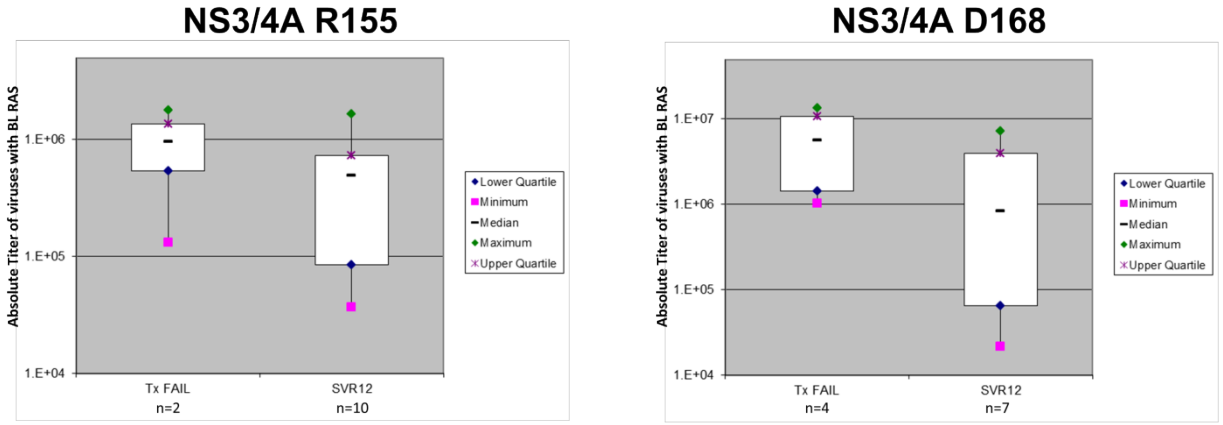


Figure 7. Absolute baseline concentration of NS3/4A R155 and D168 RAS (DAVP analysis).

For NS5A, only RAS positions with baseline substitutions in the NS5A of the HCV GT1a for subjects who failed treatment (n=10) and achieved SVR12 (n=97) were compared (Figure 8). These positions included K24, M28, Q30, L31, H58, and Y93.

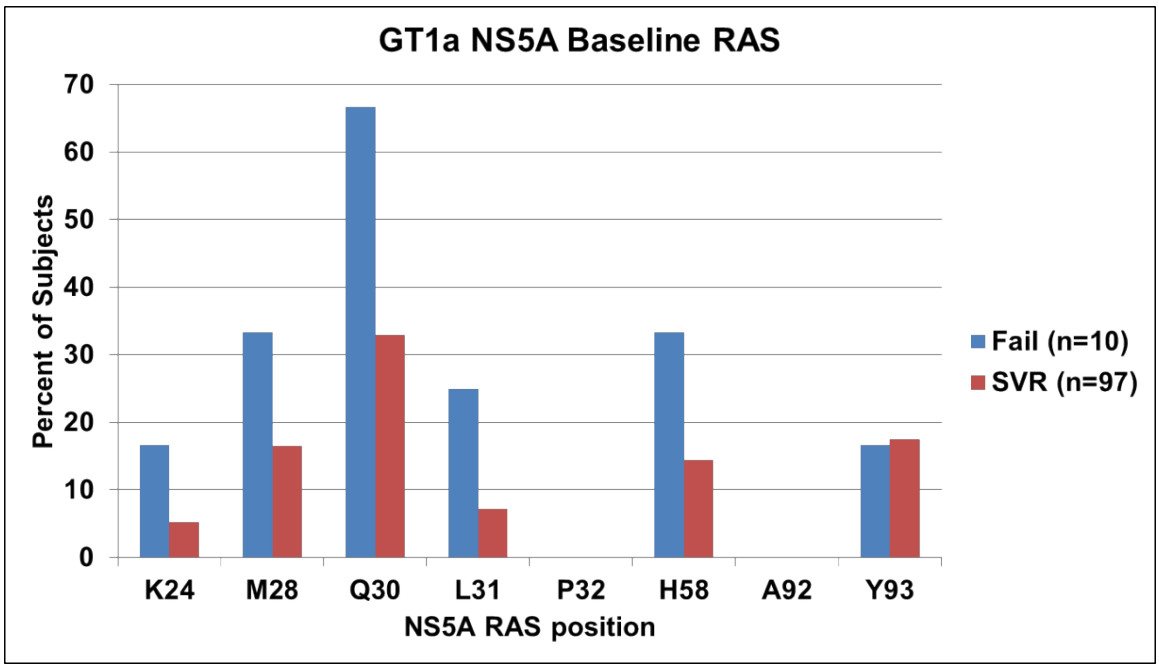


Figure 8. Baseline amino acid substitutions at RAS positions in NS5A compared between subjects infected with HCV GT1a who were treated with GLE/PIB and achieved SVR12 and those who failed treatment (DAVP Analysis). There were 10 subjects who failed treatment and 97 subjects who achieved SVR12.

For positions K24, M28, Q30, L31, and H58 the absolute concentration at baseline trended higher in subjects who failed treatment, but with overlapping ranges with those subjects who achieved SVR12 (Figure 9). Interestingly, the absolute concentration at position Y93 was similar among both groups (Figure 9).

This analysis of absolute baseline concentration of RAS indicated that subjects who failed had higher overall concentrations of known NS3/4A substitutions at positions V55 and V56 with concentrations at least 1 log_{10} higher in those who failed. For GT1a NS5A, subjects who failed had higher overall concentrations of known NS5A RAS and the median absolute concentration of M28 and H58 were ~1 log_{10} higher in those who failed, although confidence intervals overlapped with subjects who achieved SVR12 (Figure 9).

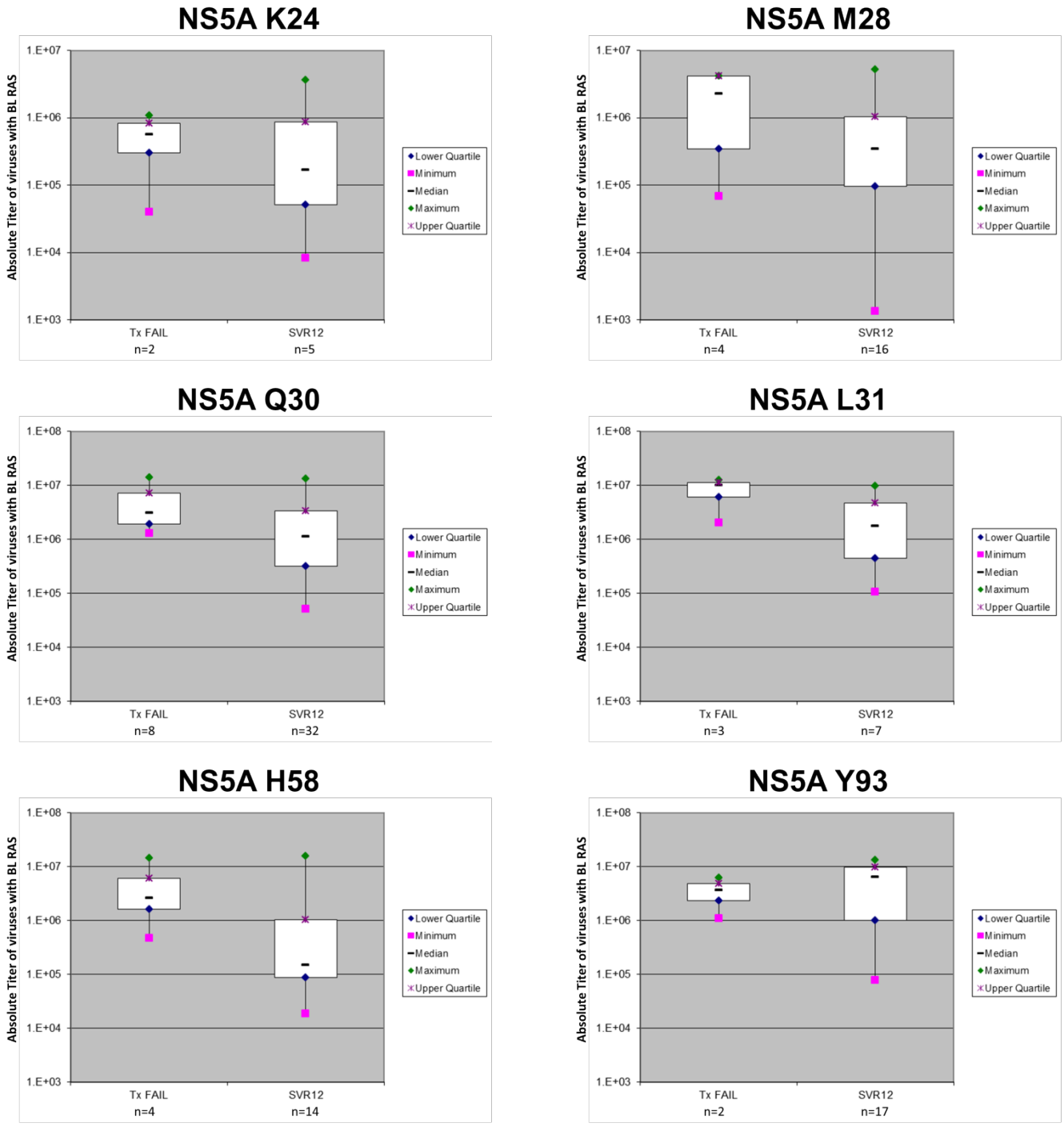


Figure 9. Absolute baseline concentrations of HCV GT1a NS5A RAS (DAVP analysis).

Of note, this baseline analysis of RAS absolute concentration looked only at single substitutions and many of the subjects who failed treatment had multiple NS5A RAS at baseline. In addition, this analysis only focused on one gene (NS3/4A or NS5A, not both). It is likely that other RAS present at baseline would add a combinatorial impact on absolute concentration at baseline and its correlation to treatment failure.

MAGELLAN-1 (M15-410) Virologic Failure Analyses

For Magellan-1 (M15-410) Part 1, the overall SVR12 rates were >86% in all treatment arms and only 2 subjects (1 each in Arms B and C, both of whom were NS5A + PI experienced) experienced virologic failure. The virologic failure rate among those with available SVR12 data in Arms B and C was the same at 4.5% (1/22), for which the sponsor concluded that the presence of RBV in the regimen does not improve efficacy.

For MAGELLAN-1 (M15-410) Part 2, the primary efficacy endpoint was the percentage of subjects who achieved SVR12 (HCV RNA <LLOQ 12 weeks after the last actual dose of study drug). Arm D assessed GLE/PIB (300/120 mg) for 12 weeks and Arm E assessed GLE/PIB (300/120 mg) administered for 16 weeks. Overall SVR12 rates were >88% in both treatment arms with an SVR12 rate of 88.6% (39/44) and 91.5% (43/47) in the ITT population for Arm D and Arm E, respectively. Although the SVR12 rates were similar, the relapse rate was lower in the 16 week treatment Arm E (0%, 0/43) compared with the 12 week treatment Arm D (9.3%, 4/43). There were 5 subjects who failed treatment in Arm D (1 BT and 4 relapsers) and 4 subjects in Arm E (all BT). In addition, resistance analysis was performed for one subject in Arm B who relapsed and one subject in Arm C who experienced BT (Table 5).

Table 5. Characteristics of subjects in MAGELLAN-1 (M15-410) who failed treatment (compiled by DAVP).

USUBJID	GT	ARM	OUTCOME	DAA EXP	BL VL (RNA copies/mL)
M15-410-2601	1a	B	Relapse	TVR,DCV	1.25E+07
M15-410-2605	1a	C	BT	PTV/r,OBV,DSV	1.44E+07
M15-410-1321	1a	D	BT	PI-naïve, LDV, SOF	1.97E+06
M15-410-2624	1a	D	Relapse	SMV,LDV,SOF	4.28E+06
M15-410-2924	1a	D	Relapse	PI-naïve, DCV	2.01E+06
M15-410-3023	1a	D	Relapse	ASV, DCV, BCV	1.88E+06
M15-410-4021	1b	D	Relapse	ASV,DCV	7.20E+05
M15-410-1620	1a	E	BT	TVR,SMV,PTV/r,LDV,OBV,SOF,DSV	1.09E+06
M15-410-2021	1a	E	BT	PI-naïve, LDV, SOF	2.02E+06
M15-410-2622	1a	E	BT	PTV/r,OBV,DSV,SOF	9.95E+06
M15-410-3025	1a	E	BT	PTV/r,OBV,DSV	6.20E+06

ASV, asunaprevir; BL, baseline; BT, breakthrough; DAA, direct-acting antiviral; DCV, daclatasvir; EXP, experienced; GT, genotype; LDV, ledipasvir; OBV, ombitasvir; PI, protease inh bitor; PTV, paritaprevir; r, ritonavir; SMV, simeprevir; SOF, sofosbuvir; TVR, telaprevir; and VL, viral load.

The results of the sponsor's resistance analysis (baseline and post-treatment for virologic failures) are shown in Table 6. Of the 11 subjects who had virologic failure, all had baseline NS5A inhibitor resistance-associated substitutions, which likely contributed to failure of PIB. Six subjects had treatment-emergent RAS at NS3/4A position A156 (two with substitutions at NS3/4A position R155), which is a resistance pathway for GLE. In addition, the one subject who was infected with HCV GT1b had a P32 deletion in the NS5A gene of HCV GT1b (Table 6).

Table 6. Integrated resistance analysis: Baseline and Post-treatment sequence analysis in DAA-experienced subjects who experienced virologic failure (MAGELLAN-1) using a 2% assay cutoff (compiled by DAVP). SUBS10, emergent substitution; AADIFF, increase in frequency from BL; AAFREQ, frequency of substitution; em, emergent substitution present at baseline with frequency increase over time.

USUBJID	GT	ARM	NS3/4A				NS5A			
			EMERGENT		BASELINE		EMERGENT		BASELINE	
			SUBS10	AADIFF	BLRAP	AAFREQ	SUBS10	AADIFF	BLRAP	AAFREQ
2601	1A	B	A156V	0.91			Q30R	1	L31M H58D	1 0.26
2605	1A	C	V36M Y56H	0.07 em 0.99	Y56H D168A D168T	0.05 0.94 0.03	M28G	0.99	M28V Q30R H58C	0.03 0.98 0.99
1321	1A	D	A156V	1			Q30K Y93H Y93N	em 1 0.98 0.02	K24E M28V Q30E Q30K Q30R	0.02 0.03 0.66 0.29 0.04
2624	1A	D	R155K A156T	em 0.98 0.98	V36M V55I Q80L R155K	0.99 0.61 0.99 0.42	M28G	0.99	M28V Q30R	0.98 0.96
2924	1A	D			V55A	1			Q30R H58D	0.97 0.98
3023	1A	D					Q30G	em 0.99	Q30E Q30G	0.05 0.93
4021	**1B**	**D**			**Y56F**	**0.99**	**L28M**	**em 1**	**L28M P32-**	**0.28 0.92**
1620	1A	E	V36M	0.98	Y56H Q80K D168E	0.97 0.99 0.95	Q30K	0.96	K24Q Y93H	1 0.99
2021	1A	E	V132I A156V	0.05 1			H58D	0.99	Q30R L31M	0.99 1
2622	1A	E	V36M V36A A156G	0.05 0.05 0.89	Y56H Q80K D168A	0.99 1 0.98			M28T M28V Q30L Q30R L31M H58D	0.41 0.35 0.02 0.98 1 0.05
3025	1A	E	R155T A156V D168A	em 0.99 1 em 1	Y56H Q80K R155T D168A D168T	0.13 1 0.02 0.25 0.03	M28A H58D	0.29 0.71	Q30H Y93H	0.99 0.99

Bold indicates a different HCV GT

MAGELLAN-1 (M15-410) Resistance Conclusions from the Sponsor

The sponsor concluded that the majority of subjects with a detected baseline RAS in NS3 or NS5A achieved SVR12. In Part 1, two GT1a-infected NS5A-experienced/PI-experienced subjects, 1 each in Arms B and C, experienced virologic failure. The subject in Arm B with relapse had no baseline polymorphisms in NS3 and had L31M and H58D in NS5A. The subject in Arm C with breakthrough had Y56H and D168A/T in NS3 and had M28V, Q30R, and H58C in NS5A at baseline. Due to the limited number of failures, no clear impact of NS3 and/or NS5A baseline polymorphisms on treatment outcome was observed in Part 1 of the study.

In Part 2, the sponsor concluded that among PI-experienced/NS5A-naïve subjects, the presence of baseline RAS in NS3 and/or NS5A had no impact on treatment outcome, as all of these subjects achieved SVR12. Baseline NS5A inhibitor RAS were highly prevalent among NS5A-experienced subjects (80.6%, 50/62), whether the previous treatment was with an NS5A inhibitor alone or an NS5A inhibitor with a PI. Among the subjects in the NS5A-experienced/PI-naïve category who had baseline NS5A inhibitor RAS, 86.7% (13/15) of those in Arm D and 91.7% (11/12) in Arm E achieved SVR12. Among PI-naïve subjects, there were no subjects with protease inhibitor RAS in NS3, and there were no virologic failures among subjects without any baseline polymorphisms. Among NS5A-experienced/PI-experienced subjects, 71.4% (5/7) of subjects with baseline NS5A inhibitor resistance-

associated polymorphisms alone in Arm D achieved SVR12, while all subjects (8/8) with NS5A polymorphisms alone in Arm E achieved SVR12. In subjects experienced to both classes of inhibitors and with RAS in both targets, lower SVR12 rates (75.0%, 3/4 in Arm D, and 25%, 1/4 in Arm E) were seen compared with subjects with NS3 RAS alone, NS5A RAS alone, or no RAS (80%, 8/10 in Arm D, and 100%, 12/12 in Arm E). Although the virologic failure rate across both treatment arms was higher in subjects with multiple baseline RAS in NS5A or across both targets than in those with single NS5A RAS or no baseline RAS, the combination of RAS seen from subject to subject did not show a pattern predictive of virologic failure.

MAGELLAN-1 (M15-410) DAVP Analysis

For the most part, the DAVP analysis of NGS data from MAGELLAN-1 (M15-410) was consistent with the results reported by the sponsor (compare Table 6 and Table 7). Most of the differences between the two analyses occurred at frequencies below 2%. However, there were two major discrepancies that occurred at high frequency. One was a frequency difference of >20% and the other a resistance-associated NS5A deletion (Table 7).

Table 7. Baseline and Post-treatment sequence analysis in DAA-experienced subjects who experienced virologic failure in MAGELLAN-1 (M15-410) using a 1% assay cutoff (DAVP Analysis).

USUBJID	GT	ARM	BL VL	NS3/4A					NS5A				
				Emergent		Baseline			Emergent		Baseline		
				SUBS10	AADIFF	BL RAP	AAFREQ	ABS CONC	SUBS10	AADIFF	BL RAP	AAFREQ	ABS CONC
M15-410-2601	1a	B	1.25E+07	A156V	0 91				Q30R	0 99	L31M	1	1 25E+07
											H58D	0.26	3 25E+06
											H58N	0.02	2 50E+05
M15-410-2605	1a	C	1.44E+07	V36M	0 06	V36A	0.03	4.32E+05	M28G	0 97	M28V	0.03	4 32E+05
				Y56H	em 0 99	V36F	0.03	4.32E+05			Q30R	0.98	1.41E+07
						Y56H	0.05	7.20E+05			Q30L	0.01	1.44E+05
						D168A	0.94	1.35E+07			H58C	0.99	1.43E+07
						D168T	0.03	4.32E+05					
M15-410-1321	1a	D	1.97E+06	A156V	1				Q30K	em 1.0	K24E	0.02	3 94E+04
									Y93H	0 97	M28V	0.03	5 91E+04
									Y93N	0 02	M28T	0.01	1 97E+04
											Q30K	0.28	5 52E+05
											Q30E	0.66	1 30E+06
											Q30R	0.05	9 85E+04
M15-410-2624	1a	D	4.28E+06	R155K	em 0 95	V36M	0.99	4.24E+06	M28G	0 99	M28V	0.98	4.19E+06
				A156T	0 96	V55I	0.59	2.53E+06			Q30R	0.96	4.11E+06
						Q80L	0.99	4.24E+06					
						R155K	0.19	8.13E+05					
						D168Y	0.02	8.56E+04					
M15-410-2924	1a	D	2.01E+06			V55A	1	2.01E+06			Q30R	0.97	1 95E+06
						D168Y	0.01	2.01E+04			Q30L	0.01	2 01E+04
											H58D	0.98	1 97E+06
											H58Y	0.01	2 01E+04
M15-410-3023	1a	D	1.88E+06						Q30G	em 0 99	Q30G	0.94	1.77E+06
											Q30E	0.05	9.40E+04
M15-410-4021	**1b**	**D**	**7.20E+05**			**Y56F**	**0.99**	**7.12E+05**	**L28M**	**em 1**	**L28M**	**0.27**	**1.94E+05**
						D168Y	**0.01**	**7.19E+03**	**P32K**	**em 1**	**R30Q**	**0.02**	**1.44E+04**
											L31K	**0.02**	**1.44E+04**
											P32R	**0.04**	**2.88E+04**
											P32N	**0.18**	**1.29E+05**
											P32K	**0.7**	**5.03E+05**
											P32T	**0.08**	**5.75E+04**
M15-410-1620	1a	E	1.09E+06	V36M	0 97	Y56H	0.97	1.06E+06	K24R	0.18	K24Q	1	1 09E+06
						Q80K	0.98	1.07E+06	Q30K	0 95	Y93H	0.99	1 08E+06
						D168E	0.95	1.04E+06					
M15-410-2021	1a	E	2.02E+06	V132I	0 05				H58D	0 99	Q30R	0.99	2 00E+06
				A156V	1						L31M	1	2 02E+06
M15-410-2622	1a	E	9.95E+06	V36M	0 04	Y56H	0.99	9.85E+06			M28V	0.35	3.48E+06
				V36A	0 04	Q80K	1	9.95E+06			M28T	0.41	4 08E+06
				A156G	0 89	D168A	0.98	9.75E+06			Q30R	0.97	9 65E+06
											Q30L	0.02	1 99E+05
											L31M	1	9 95E+06
											H58D	0.05	4 98E+05
M15-410-3025	1a	E	6.20E+06	R155T	em 0 99	Y56H	0.13	8.06E+05	M28A	0 29	M28V	0.01	6 20E+04
				A156V	1	Q80K	0.99	6.14E+06	H58D	0.7	Q30H	0.99	6.14E+06
						R155T	0.01	6.20E+04			Y93H	0.99	6.14E+06
						D168A	0.25	1.55E+06					
						D168T	0.04	2.48E+05					

SUBJID, subject ID; **GT**, HCV genotype; **Emergent**, indicates an increase in RAS frequency at the post-treatment timepoint; **SUBS10**, is the emergent RAS detected; **AADIFF**, is the different in frequency of the emergent RAS as compared to baseline; **BL RAS**, RAS detected at baseline; **AAFREQ**, the frequency of the baseline RAS; **em**, emerged RAS that increased from BL to post-treatment timepoint. **Yellow**, indicates discrepancies between the sponsor's variant calls or frequencies and those detected by the DAVP analysis pipeline.

These discrepancies were likely related to the different analysis approaches used by DAVP and by the sponsor. To provide additional information for these regions of disagreement, a third analysis pipeline was used to assess the fastq sequences derived from the two subjects (2624 and 4021). These positions were analyzed using the CLC Genomics workbench which was in agreement with the results reported by the sponsor. For the HCV NS3/4A sequences derived from subject 2624, the different analysis pipelines resulted in different coverages at position R155, which likely contributed to the discrepancy (Table 8).

Table 8. Differences in coverage and frequency at NS3/4A position R155 in the HCV of subject 2624 (DAVP analysis).

Analysis pipeline	Coverage at R155K	Frequency R155K detected at:
Sponsor	18,952	0.42
HIVE	13,811	0.19
CLC Genomics	15,991	0.42

This discrepancy highlights the concerns of the division in reviewing NGS data as differences in algorithms and parameters resulted in the difference. Given the nondeterministic design of some components of any NGS analysis pipeline, an independent assessment of the NGS data using a second and/or third approach is essential for assessing the reliability of the results.

For the NS5A P32 deletion reported by the sponsor but called an amino acid substitution by HIVE, CLC Genomics analysis supported the sponsor's results. HIVE did not report this as a deletion, but did report the amino acid adjacent as a substitution. This error will be addressed with the HIVE team.

MAGELLAN-1 (M15-410) DAVP conclusions: In general, the NGS analysis performed by the sponsor/CRO was robust and reproducible. There was good agreement between their analysis pipeline and the one used by DAVP. Of the 11 subjects who had virologic failure, all had baseline NS5A inhibitor resistance-associated substitutions, which likely contributed to failure of PIB. Six subjects had treatment-emergent RAS at NS3/4A position A156 (two with substitutions at NS3/4A position R155), which is a resistance pathway for GLE. Two major discrepancies (a large frequency difference and a P32 deletion) were determined to be algorithm-specific errors, highlighting the importance of conducting an independent assessment of NGS data. No novel resistance pathways were identified; however, baseline RAS in HCV GT1a at NS5A position P77 and NS3/4A positions T61, S189, V490, V535, and I668 could potentially be associated with poor treatment outcomes. Absolute concentrations of baseline resistance-associated substitutions may play a role in treatment failure; however, given the complex mutational profile of DAA-experienced subjects in this trial and the small samples sizes, no conclusions could be drawn on these data.

CONCLUSIONS

DAVP performed an independent analysis of the NGS data submitted for the phase 2 clinical trial, MAGELLAN-1 (M15-410), and compared the results to those reported by the sponsor. In general, there was good agreement between these results. However, the different analysis pipelines used much different default filtering and mapping criteria and this was apparent when comparing frequency table values such as Total Coverage, Variant Coverage, and Amino Acid Frequency. However, despite these differences, the general trends observed were very similar and there was generally good agreement at frequencies greater than 2%. Two major discrepancies (a large frequency difference and a P32 deletion) were determined to be algorithm-specific errors and a third NGS analysis was performed for the sequence read files for which the discrepancies were observed. The third algorithm provided support for the sponsor's results. These discrepancies highlight the concerns of the division in reviewing NGS data as differences in algorithms and/or parameters resulted in the difference. Given the nondeterministic design of some components of any NGS analysis pipeline, an independent

assessment of the NGS data using a second and/or third approach is essential for confirming the reliability of the results.

No novel treatment-emergent resistance pathways were identified; however, baseline RAS in HCV GT1a at NS5A position P77 and NS3/4A positions T61, S189, V490, V535, and I668 could potentially be associated with poor treatment outcomes. These positions were reported by the sponsor but no phenotypic data were provided. All substitutions meeting the SUBS10 criteria are shown in Appendix II. Emerging RAS that increased in frequency from baseline to the posttreatment timepoint are shown in Appendix III.

Absolute concentrations of baseline resistance-associated substitutions may play a role in treatment failure; however, given the complex mutational profile of the HCV in DAA-experienced subjects in this trial and the small samples sizes, no conclusions could be drawn on these data.

The NGS analyses did not identify any issues that would impact labeling. For complete labeling details, please see the review of NDA 209394 SDN 000 by Senior Clinical Virology Reviewer Patrick Harrington, Ph.D.

POST MARKETING RECOMMENDATIONS

(b) (4)

ADMINISTRATIVE

Reviewer's Signature(s)

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

Concurrence(s)

_____Date: _____
HFD-530/Clin Micro TL/J O'Rear

cc:
HFD-530/NDA
HFD-530/Division File
HFD-530/RPM/Gentles

APPENDICES

I. METHODS (Copied from the NDA)

Deep Sequencing Assays

Deep sequence analysis of HCV comprises the following steps

RNA isolation
RNA is isolated from plasma or serum using e.g., the BioMerieux EasyMag, the QIAamp MinElute Virus method (Qiagen) or the Roche MagNA Pure 96 instrument. A well-characterized sample, containing a known viral load, is used as positive control. As a standard procedure, RNA is isolated from 200-500 µL of serum, and extracted RNA is eluted in 20-50 µL (depending on the isolation method), but other volumes are possible.

cDNA synthesis
HCV viral RNA is reverse transcribed into cDNA using the Roche Transcriptor kit (Roche Molecular Systems), employing specific antisense primers. 10 µL of isolated RNA is used for cDNA synthesis.

PCR and nested PCR
The cDNA is amplified by target specific PCR and nested PCR, using the Expand high-fidelity PCR kit (Roche Molecular Systems). 10 µL of cDNA is used for PCR.

Analysis of amplified products by gel-electrophoresis
PCR products are analyzed by gel-electrophoresis or a capillary system (Qiaxcel from Qiagen) using standard protocols, to confirm successful amplification of a PCR fragment of the expected size, and to determine whether the quantity of product is sufficient for successful deep sequence analysis.

Size selection by Ampure XP bead purification
The PCR products that show a clear band of the expected size after gel-electrophoresis will be cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small aspecific PCR products. PCR products will not be extracted from gel due to high risk of contamination.

Quantification and dilution of dsDNA
The purified PCR products will be quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies), followed by dilutions and (optional) pooling of targets.

Library preparation
The diluted PCR products are fragmented and tagged by using the 'tagmentation' method. Index primers will be added by limited cycle PCR. Prior to library pooling, samples are normalized by using beads with maximum binding capacity.

Sequencing
Sequencing will be performed on the Illumina Miseq platform by using a Miseq sequencing kit (generally v2, 300 cycle chemistry). De-multiplexed fastq files will be generated as an output.

QC and Variant calling
Several QC parameters are deduced from the fastq reads. General QC parameters reporting options are: total number of reads, % mapped to reference, % Q(b) (4) average read length, minimum coverage, average coverage and maximum coverage.

When the fastq reads are used for generation of RAV (Resistance-Associated Variants) reports, they are Quality trimmed and mapped to a general reference (first mapping). The consensus sequence extracted from this first mapping is used as a reference for a second mapping. This mapping is used to

perform quality based variant calling. Variant calling is performed in the (b) (4) Athena pipeline. The (b) (4) Athena pipeline (including the generated reports) is described in a separate below.

General description Athena pipeline

This document contains a general description of the Athena pipeline for analysis of deep sequencing data, as well as a description of the resulting report.

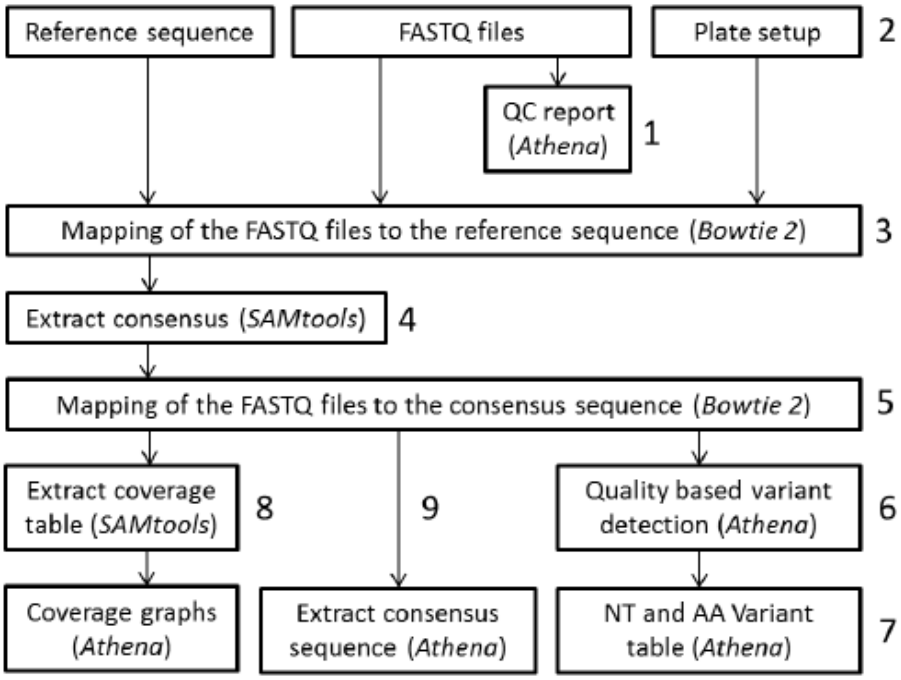


Figure A1. Flowchart on the steps of the Athena pipeline (Figure 1, Appendix A, RD161026 NGS Methods).

The Athena pipeline comprises the following steps:

1. Fastq files, generated by the Illumina MiSeq, will be imported in Athena. Basic QC values will be determined (number of reads, average read length, % of reads with Q≥ (b) (4)).
2. Plate setup defines the targets to be analyzed for each sample.
3. Filtered fastq reads will be mapped to a general reference sequence (Bowtie2, settings described in table 4).
4. A consensus sequence for each sample will be extracted from the first mapping (SAMtools, standard settings).
5. To increase the accuracy of the mapping, the fastq reads will be remapped (second mapping) to the generated consensus sequence from step 4.
6. The individual reads within the mapping will be translated into amino acids, followed by quality based variant detection in Athena.
7. An Athena report will be generated. Generally, only the AA report is provided. If required, the NT report can also be provided.
8. From the second mapping, a coverage table can be extracted. The coverage is visualized in a coverage graph.
9. From the second mapping, a consensus sequence can be extracted. This is a full genome consensus sequence which contains 'n's if coverage is not sufficient. Insertions are annotated with lower case characters.

Table A1. QC information from the Athena report (Table 1, Appendix A, RD161026 NGS Methods).

(b) (4)

[redacted]

Reference Sequences Used for NGS Analysis (Appendix D, RD161026 NGS Methods)

Genotype	GenBank Accession	Strain
1a	NC_004102	H77
1b	AJ238799	Con1
1c	D14853	HC-G9
1d	KJ439768	QC103
1e	KJ439769	QC172
1g	AM910652	1804
1h	KC248198	EBW443
1i	KJ439772	QC181
1j	KJ439773	QC329
1k	KJ439774	QC82
1l	KC248193	136142
1m	KJ439778	QC196
1n	KJ439775	QC113
2a	AB047639	JFH-1
2b	D10988	HC-J8
2c	D50409	BEBE1
2d	JF735114	QC259
2e	JF735120	QC64
2f	KC844042	ZS542
2i	DQ155561	D54
2j	HM777359	C1292
2k	AB031663	VAT96
2l	KC197235	MRS89
2m	JF735111	QC178
2q	FN666429	852
2r	JF735115	QC283
2t	KC197238	MRS40

Reference Sequences (continued)

Genotype	GenBank Accession	Strain
3a	GU814263	S52
3b	D49374	HCV-Tr
3d	KJ470619	NE274
3e	KJ470618	NE145
3g	JX227954	BID-G1243
3h	JF735121	QC29
3i	FJ407092	IND-HCV-3i
3k	D63821	JK049
4a	GU814265	ED43
4b	FJ462435	QC264
4c	FJ462436	QC381
4d	FJ462437	QC382
4f	EF589161	IFBT88
4g	FJ462432	QC193
4k	FJ462438	QC383
4l	FJ839870	QC274
4m	FJ462433	QC249
4n	FJ462441	QC97
4o	FJ462440	QC93
4p	FJ462431	QC139
4q	FJ462434	QC262
4r	FJ462439	QC384
4t	FJ839869	QC155
4v	HQ537008	CYHCV048
5a	AF064490	SA13
6a	Y12083	EUHK2
6b	NC_009827	Th580
6c	EF424629	Th846
6d	D84263	VN235
6e	DQ314805	GX004
6f	DQ835760	C-0044
6g	DQ314806	HK6554
6h	D84265	VN004
6i	DQ835762	C-0159
6j	DQ835769	Th553
6k	DQ278893	KM41
6l	EF424628	537796
6m	DQ835765	C-0185
6n	AY878652	KM42
6o	EF424627	QC227
6p	EF424626	QC216
6q	EF424625	QC99
6r	EU408328	QC245
6s	EU408329	QC66
6t	EU246939	D49
6u	EU246940	D83
6v	EU798761	KM046
6w	EU643836	HCV-6-D370
6xa	EU408330	DH012
6xb	JX183552	TV476
6xc	KJ567651	6_TV520
6xd	KM252791	L394
6xe	JX183557	DH027
7a	EF108306	QC69

Sequencing-based HCV Subtype Determination

The Versant HCV Genotype Inno-LiPA Assay v2.0 (LiPA 2.0), conducted by the Central Lab, is used to determine HCV genotype for enrollment of subjects into Phase 2 and 3 clinical studies. However, for

HCV Genotypes 2, 4, and 6 the LiPA 2.0 assay is unable to accurately identify the viral subtype. Therefore, for instances where a viral subtype cannot be determined by LiPA 2.0, the viral subtype is determined by phylogenetic analysis of a 329 nucleotide region of NS5B that is PCR amplified from the baseline samples (with HCV RNA ≥1,000 IU/mL) of HCV-infected subjects. Results from the NS5B phylogenetic analysis are used to assign a preliminary subtype to each HCV-infected subject sample with an ambiguous subtype, which then determines the subtype-specific reverse transcriptase (RT)-PCR and nested PCR primer sets for independent amplification of NS3/4A and NS5A genes from baseline samples.

The subtype-specific RT-PCR, nested PCR, and sequencing primers were designed at AbbVie and were based on the alignments of available sequences in GenBank and the European HCV database. The primers were designed in conserved regions specific to the gene of interest, and nucleotide degeneracies were incorporated in positions where significant variability existed among the sequences. The target genes are amplified from HCV RNA by RT-PCR followed by nested P-CR using subtype specific primers encoding NS3/4A protease or NS5A. Only samples with ≥1,000 IU/mL HCV RNA are amplified in order to reduce the chances of oversampling bias. For samples with ≤50,000 IU/mL HCV RNA, the RT-PCR is done in triplicate and the products are pooled prior to their use as a template for nested PCR.

Nested PCR products encompassing the genes encoding full-length NS3/4A or NS5A are generated by AbbVie Clinical Virology and sent for NGS analysis to be performed by either (b) (4)

II. NOVEL RAS MEETING SUBS10 CRITERIA Detected By DAVP

HCV GT	HCV Pro	USUBJID	AAPOS	AADIFF	SUBS10	Conservation
1a	NS34A	M15-410-1321	77	0.52	N77S	Conserved
		M15-410-2624	116	0.64	V116A	Conserved
		M15-410-2601	347	0.13b	V347I	
		M15-410-3025	360	0.3	K360N	
		M15-410-2622	482	0.84	P482S	
		M15-410-2622	629	0.11b	I629V	
		M15-410-2601	630	0.1	V630F	
		M15-410-1620	654	0.96	V654A	Conserved
		M15-410-2924	678	0.13b	Q678E	
	NS5A	M15-410-3023	29	1	P29R	
		M15-410-2924	37	0.27	V37Mb	
		M15-410-1620	61	0.72	A61T	Conserved
		M15-410-2624	79	1	T79A	
		M15-410-2624	122	0.92	R122Kb	
		M15-410-2021	143	0.18	Q143R	
		M15-410-3025	223	0.24	P223H	
		M15-410-2021	240	0.12	K240Rb	
		M15-410-2021	273	0.12	E273G	
		M15-410-2601	293	0.2	E293G	
		M15-410-1321	305	0.49	K305R	
		M15-410-3025	310	0.99	A310V	
		M15-410-3025	357	0.73	K357R	
		M15-410-3025	383	0.74	S383F	
		M15-410-1620	389	0.1	T389A	
		M15-410-1321	392	0.18	N392D	
		M15-410-1321	395	0.11	T395M	
		M15-410-2601	402	0.52	S402P	
		M15-410-2624	422	0.98	E422G	
		M15-410-2601	431	0.19	G431R	
1b	NS34A	None				
	NS5A	None				

HCV GT, HCV genotype; **HCV Pro**, HCV protein that contains the substitution; **USUBJID**, unique subject ID; **AAPOS**, amino acid position; **AADIFF**, difference in frequency from baseline to posttreatment timepoint; **SUBS10**, amino acid substitution that met the SUBS10 criteria; **Conservation**, substitutions that occurred at highly conserved positions defined as those amino acid sites in the HCV protein from subjects in the general treatment population where substitutions were detected in 1% or less of subjects.

III. EMERGENT RAS Detected by DAVP

HCV GT	HCV Pro	USUBJID	AAPOS	SUBS	AADIFF	BLFREQ	TFFREQ	Conservation
1a	NS34A	M15-410-2622	18	I18V	0.88	0.08	0.96	Conserved
		M15-410-2924	18	I18V	0.77	0.23	1	Conserved
		M15-410-2622	117	R117C	0.45	0.42	0.87	
		M15-410-3025	174	N174G	0.39	0.58	0.97	
		M15-410-3023	329	V329I	0.94	0.06	1	
		M15-410-2624	354	I354V	0.68	0.27	0.95	Conserved
		M15-410-3025	358	V358A	0.26	0.74	1	
		M15-410-2924	660	I660V	0.78	0.22	1	
1a	NS5A	M15-410-2624	78	R78K	0.94	0.01	0.95	
		M15-410-2601	107	K107E	0.95	0.03	0.98	
		M15-410-1620	251	D251N	0.57	0.09	0.66	
		M15-410-2624	280	I280V	0.97	0.03	1	
		M15-410-2624	400	A400T	0.98	0.02	1	

HCV GT, HCV genotype; **HCV Pro**, HCV protein that contains the substitution; **USUBJID**, unique subject ID; **AAPOS**, amino acid position; **SUBS**, substitution detected; **AADIFF**, difference in frequency from baseline to posttreatment timepoint; **BLFREQ**, baseline frequency; **TFFREQ**, frequency detected at the posttreatment timepoint; **Conservation**, substitutions that occurred at highly conserved positions defined as those amino acid sites in the HCV protein from subjects in the general treatment population where substitutions were detected in 1% or less of subjects.

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/s/

ERIC F DONALDSON
05/11/2017

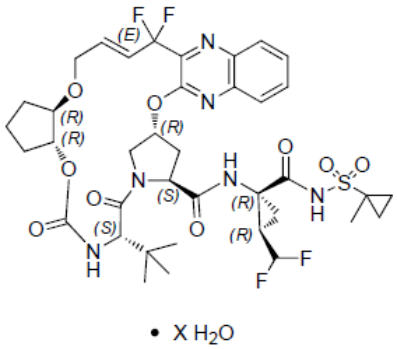
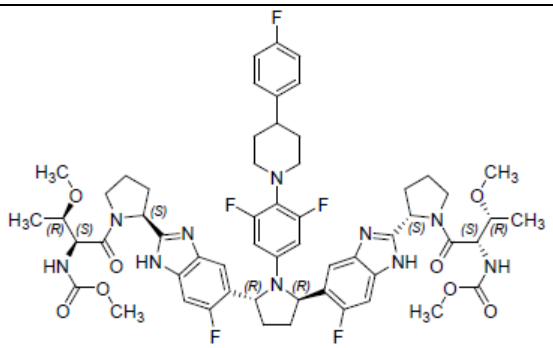
JULIAN J O REAR
05/11/2017

DIVISION OF ANTIVIRAL PRODUCTS
CLINICAL VIROLOGY REVIEW
NDA: 209394 **SDN**: 000 (Original NDA, SDN 1 in DARRTS) **REVIEW COMPLETED:** 05/05/2017
Clinical Virology Reviewer: Patrick R. Harrington, Ph.D.

NDA#: 209394 **SDN:** 000 (Original NDA)
Reviewer's Name(s): Patrick R. Harrington, Ph.D.

Sponsor: AbbVie, Inc.
1 N. Waukegan Road
North Chicago, IL 60064
Sejal P. Emerson, PharmD
Director, Regulatory Affairs

Initial Complete Submission Dates:
Correspondence Date: 12/14/2016
CDER Receipt Date: 12/14/2016
Assigned Date: 12/15/2016
Review Complete Date: 05/05/2017
PDUFA Date: 8/14/2017

Proprietary Name (tentative)	**Mavyret™ (fixed dose combination product: glecaprevir/pibrentasvir)**	
Individual Drug Names [class]	glecaprevir (GLE, ABT-493) [NS3/4A protease inhibitor]	pibrentasvir (PIB, ABT-530) [NS5A inhibitor]
Parent IND #s	116169 (GLE), 127416 (GLE/PIB)	116170 (PIB), 127416 (GLE/PIB)
Chemical Names	(3a*R*,7*S*,10*S*,12*R*,21*E*,24a*R*)-7-*tert*-butyl-*N*-{(1*R*,2*R*)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl] cyclopropyl}-20,20-difluoro-5,8-dioxo-2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1*H*,10*H*-9,12-methanocyclopenta[18,19] [1,10,17,3,6]trioxadiazacyclononadecino[11,12-*b*]quinoxaline-10-carboxamide hydrate (IUPAC)	Methyl {(2*S*,3*R*)-1-[(2*S*)-2-{5-[(2*R*,5*R*)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)piperidin-1-yl]phenyl}-5-(6-fluoro-2-{(2*S*)-1-[*N*-(methoxycarbonyl)-*O*-methyl-L-threonyl]pyrrolidin-2-yl}-1*H*-benzimidazol-5-yl)pyrrolidin-2-yl]-6-fluoro-1*H*-benzimidazol-2-yl}pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl}carbamate (IUPAC)
Structures	 • X H_2O **GLECAPREVIR**	 **PIBRENTASVIR**
Molecular Formulas	$C_{38}H_{46}F_4N_6O_9S$ (anhydrate)	$C_{57}H_{65}F_5N_{10}O_8$
Molecular Weights	838.87 (anhydrate)	1113.18

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Amendments: none

Related/Supporting Documents: Additional/updated information submitted in the following SDNs: 4 (response to Clinical Virology request), 5 (resistance data from ENDURANCE-3 control arm), 7 (response to Clinical request), 9 (response to Clinical Virology request), 10 (response to Clinical request), 18 (response to Clinical Virology request), 22 (response to mid-cycle comments), 23 (MAGELLAN-1 prior regimen dataset), 25 (sponsor's mid-cycle meeting minutes), 26 (4-month safety update), 31 (response to labeling comments sent 4/7/17)

Dosage Form and Route of Administration: 100 mg glecaprevir and 40 mg pibrentasvir fixed-dose combination tablet; Oral (3 tablets/day)

Dispensed: Rx x OTC__

Proposed Indication(s): Treatment of patients with chronic HCV genotype (GT) 1, 2, 3, 4, 5 or 6 infection

Abbreviations: ADR, adverse drug reaction; ASV, asunaprevir; BCV, beclabuvir; BOC, boceprevir; CC, cytotoxicity concentration; CI, confidence interval; CSR, clinical study report; DAA, direct-acting antiviral agent; DCV, daclatasvir; DLV, deleobuvir; DSV, dasabuvir; EC, effective concentration; ESRD, end-stage renal disease; FAL, faldaprevir; FDC, fixed-dose combination; GLE, glecaprevir; HCV, hepatitis C virus; GT, genotype; HBV, hepatitis B virus; HIV(-1), human immunodeficiency virus (type 1); IC, inhibitory concentration; IFN(α), interferon (alfa); ITT, intent-to-teat; LDV, ledipasvir; LiPA, line-probe assay; LLOQ, lower limit of quantification; ND, no data; NGS, next generation sequencing; OBV, ombitasvir; ODV, odalasvir; Peg-IFNα, pegylated interferon α; PI, protease inhibitor; PIB, pibrentasvir; P/R, pegylated interferon α plus ribavirin; P/R/S, regimens containing interferon, pegylated interferon, ribavirin, and/or sofosbuvir; PTV/r, paritaprevir/ritonavir; RAS, resistance-associated substitution; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SMV, simeprevir; SOF, sofosbuvir; SVP, sovaprevir; SVR, sustained virologic response; TE, treatment-experienced; TN, treatment-naïve; TVR, telaprevir; VDV, vedroprevir; VF, virologic failure;

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EXECUTIVE SUMMARY

1. RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

This Original NDA for glecaprevir/pibrentasvir is approvable from a Clinical Virology perspective for the treatment of chronic HCV genotype 1-6 infection, including treatment-naïve patients and also patients with prior treatment experience with interferon (or pegylated interferon), ribavirin, or sofosbuvir, and patients with or without compensated cirrhosis. In addition, the NDA is approvable for the treatment of HCV genotype 1 infected patients who have prior treatment experience with an NS3/4A protease inhibitor or an NS5A inhibitor; however, this reviewer does not recommend approval for the treatment of HCV genotype 1 infected patients with prior treatment experience with both of these drug classes due to a higher risk of treatment failure and drug resistance selection. In addition, this reviewer does not recommend approval for patients with non-genotype 1 infection who have prior treatment experience with an NS3/4A protease inhibitor or an NS5A inhibitor, due to lack of sufficient efficacy data in this population.

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

This reviewer recommends the following post-marketing commitments or requirements (as appropriate):

1) (b) (4)

2)

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

Glecaprevir
Glecaprevir (GLE) is an HCV NS3/4A protease inhibitor (PI) with broad HCV genotypic activity. In biochemical assays, GLE inhibited the activity of recombinant NS3/4A protease enzymes from HCV genotype/subtypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a, with IC_{50} values of 3.5-11.3 nM. In cell culture assays, GLE inhibited the replication of HCV replicons carrying NS3 protease genes from HCV subtypes 1a, 1b, 2a, 2b, 3a, 4a and 6a, with EC_{50} values of 0.85-4.6 nM, and a therapeutic index of approximately 85,000. GLE also had consistent, sub- to low nanomolar activity against a panel of transient HCV replicons derived from clinical isolates. In cell culture combination studies, GLE had non-antagonistic antiviral activity with pibrentasvir (PIB, NS5A inhibitor), sofosbuvir (SOF, uridine nucleotide analogue NS5B polymerase inhibitor) and ribavirin (RBV).

In cell culture resistance selection studies, GLE exposure of HCV replicons from HCV subtypes 1a, 1b, 3a, 4a or 6a resulted in the emergence of amino acid substitutions in NS3. The most common substitutions observed occurred at NS3 amino acid position A156, which is a known NS3/4A PI resistance-associated position. The selection of amino acid substitutions at this position as well as others (including Y56 and D/Q168) indicates that GLE has at least partially overlapping resistance mechanisms with other HCV NS3/4A PIs.

Analyses of HCV replicons engineered to carry specific NS3 amino acid substitutions demonstrated that GLE maintained activity against replicons harboring individual amino acid substitutions at certain NS3 positions commonly associated with resistance to other NS3/4A PIs, including positions V36, Y56, Q80 and R155. However, GLE activity was reduced by amino acid substitutions at NS3 positions A156 and D168 (or Q168 for GT3), in some cases by as much as >1,000-fold, consistent with the emergence of substitutions at these positions in GLE-selected replicons. Certain combinations of amino acid substitutions including these positions further reduced GLE anti-HCV activity.

Pibrentasvir

Pibrentasvir (PIB) is an HCV NS5A inhibitor with broad HCV genotypic activity. PIB inhibited the replication of HCV replicons carrying NS5A genes from genotype/subtypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a, with EC_{50} values of 1.4-5 pM, and a therapeutic index >10,000,000. PIB was also shown to have consistent, sub- to low pM activity against a panel of transient HCV replicons derived from clinical isolates. In cell culture combination studies, PIB had non-antagonistic antiviral activity with other anti-HCV agents including GLE (NS3/4A PI), SOF (uridine nucleotide analogue NS5B polymerase inhibitor), and RBV.

In cell culture resistance selection studies, PIB exposure of HCV GT1a or GT1b replicons resulted in the emergence of amino acid substitutions in NS5A of GT1a replicons, but no PIB-resistant GT1b replicons were selected. PIB-resistant GT1a replicons primarily carried an NS5A Y93H substitution, while a few clones had emergent NS5A Q30D, Q30-deletion, Y93N, or H58D + Y93H substitutions. All of these substitutions occurred at known NS5A inhibitor resistance-associated positions, confirming that PIB is an NS5A inhibitor, and also indicating that PIB has at least partially overlapping resistance mechanisms with other NS5A inhibitors. Similar PIB resistance selection and characterization studies were conducted for replicons harboring NS5A genes from non-GT1 isolates, which again showed resistance selection at known resistance-associated positions, although in general few drug-resistant clones were obtained.

Analyses of HCV replicons engineered to carry specific NS5A amino acid substitutions demonstrated that PIB maintained activity against replicons harboring various single amino acid substitutions associated with resistance to NS5A inhibitors. Among all of the site-directed mutant replicons evaluated across all HCV GTs, the only single NS5A amino acid substitutions that reduced PIB activity ≥10-fold were M28G and Q30D in GT1a, and P29-deletion and P32-deletion in GT1b. Nevertheless, with the exception of the P32-deletion PIB remained active at picomolar levels against these replicons. The presence of two or more NS5A inhibitor resistance-associated substitutions had a greater impact on PIB activity, indicating that combinations of substitutions can act synergistically to confer resistance to PIB.

2.2 Clinical Virology

Overview of Clinical Trials

The GLE/PIB NDA is supported by efficacy, safety, and resistance data from 9 Phase 2b or Phase 3 clinical trials that included approximately 2,400 subjects who received GLE and PIB at the to-be-marketed dose levels (Table 1). Study subjects enrolled across these trials represented a broad range of HCV patient populations, including all major HCV GTs (1-6), treatment-naïve and treatment-experienced patients (including experience with interferon, sofosbuvir, NS3/4A PIs or NS5A inhibitors), patients with or without compensated cirrhosis, HCV/HIV-1 coinfected patients, and patients with renal impairment.

Efficacy and resistance analyses generally were conducted according to subjects' HCV genotype and whether they had prior treatment experience with HCV NS3/4A PIs or NS5A inhibitors. Eight of the 9 reviewed trials included subjects without prior NS3/4A PI or NS5A inhibitor treatment experience, although subjects with prior interferon (IFN)- or SOF-based treatment experience were eligible for 7 of these trials. Some of the trial

designs for NS3/4A PI- and NS5A inhibitor-naïve subjects did not allow for direct comparison of different regimens (e.g., 8-week vs. 12-week duration) for the same HCV patient population within the same trial, and therefore pooled analyses across trials were conducted for efficacy and resistance analyses. The single trial that included subjects with prior NS3/4A PI or NS5A inhibitor experience, MAGELLAN-1 (M15-410), was reviewed separately.

Table 1. Overview of key Phase 2b/3 trials of GLE/PIB. Abbreviations: DCV, daclatasvir; ESRD, end-stage renal disease; P/R, pegylated interferon α plus ribavirin; P/R/S, regimens containing any interferon, pegylated interferon, ribavirin, and/or sofosbuvir (excl. other DAAs); SOF, sofosbuvir; TE, treatment-experienced; TN, treatment-naïve;

Trial Number (Trial Name w/ clinicaltrials.gov link)	Clinical Phase	Population	Regimen(s)
M13-590 (ENDURANCE-1)	Phase 3	GT1, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 8W or 12W
M15-464 (ENDURANCE-2)	Phase 3	GT2, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 12W or Placebo
M13-594 (ENDURANCE-3)	Phase 3	GT3, noncirrhotic, TN	GLE/PIB 8W or 12W, DCV+SOF 12W
M13-583 (ENDURANCE-4)	Phase 3	GT4-6, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 12W
M14-172 (EXPEDITION-1)	Phase 3	GT1,2,4-6, compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB 12W
M15-462 (EXPEDITION-4)	Phase 3	GT1-6, renal imp./ESRD, noncirrhotic or compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB 12W
M14-868 (SURVEYOR-2)	Phase 2b	GT2-6, noncirrhotic or compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB ± RBV 8-16W
M14-867 (SURVEYOR-1)	Phase 2b	GT1,4-6, noncirrhotic or compensated cirrhosis, TN or TE (P/R)	GLE/PIB 8 or 12W
M15-410 (MAGELLAN-1)	Phase 2b	GT1,4, noncirrhotic or compensated cirrhosis, NS3/4A PI- or NS5A inhibitor-experienced	GLE/PIB 12W or 16W

For all trials the primary efficacy variable was sustained virologic response (HCV RNA below lower limit of quantification, <LLOQ) at Post-Treatment Week 12 (SVR12). Genotypic resistance analyses were conducted primarily by using a next generation nucleotide sequencing (NGS) assay. For detailed NGS results and details on technical aspects of the NGS analyses, please see the Clinical Virology review by Dr. Eric Donaldson. The minimum NGS sensitivity cutoff for data reporting was 2%, although most of the key resistance analyses conducted for this review used a 15% NGS sensitivity cutoff, which is comparable to Sanger population nucleotide sequencing and considered more clinically meaningful.

Pooled Efficacy Summary for NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

Among HCV GT1-6 subjects without prior NS3/4A PI or NS5A inhibitor treatment experience (8 of the 9 reviewed trials), GLE/PIB treatment for 8-16 weeks resulted in high SVR12 rates, often approaching 100%. Table 2 (FDA analysis) provides a pooled summary of SVR12 and virologic failure (VF) rates across all Phase 2b/3 trials that included NS3/4A PI- and NS5A inhibitor-naïve subjects.

Table 2. Pooled SVR12 and virologic failure results for NS3/4A PI-naïve and NS5A inhibitor-naïve subjects (i.e., excluding MAGELLAN-1/M15-410) who received GLE/PIB to-be-marketed dose levels in Phase 2b/3 trials. Highlighted rows show data for regimens initially proposed by the sponsor for the label.

HCV GT	Cirrhosis?	Tx History	Regimen	SVR12	VF Rate	On-Tx VF	Relapse	Non-VF
GT1	N	Naïve	GLE/PIB 8W	99% (245/248)	0% (0/248)	0	0	3
			GLE/PIB 12W	99.6% (241/242)	0% (0/242)	0	0	1
		IFN- or P/R-exp	GLE/PIB 8W	99% (137/138)	1% (1/138)	1	0	0
			GLE/PIB 12W	100% (155/155)	0% (0/155)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 8W	1/1	0/1	0	0	0
			GLE/PIB 12W	100% (4/4)	0% (0/4)	0	0	0
	Y	Naïve	GLE/PIB 12W	97% (69/71)	0% (0/71)	0	0	2
		IFN- or P/R-exp	GLE/PIB 12W	96% (24/25)	4% (1/25)	0	1	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (5/5)	0% (0/5)	0	0	0
GT2	N	Naïve	GLE/PIB 8W	99% (172/174)	0% (0/174)	0	0	2
			GLE/PIB 12W	99% (167/169)	0% (0/169)	0	0	2
		IFN- or P/R-exp	GLE/PIB 8W	94% (16/17)	6% (1/17)	0	1	0
			GLE/PIB 12W	100% (60/60)	0% (0/60)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 8W	83% (5/6)	17% (1/6)	0	1	0
			GLE/PIB 12W	100% (5/5)	0% (0/5)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (26/26)	0% (0/26)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (3/3)	0% (0/3)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
GT3	N	Naïve	GLE/PIB 8W	95% (177/186)	3% (6/186)	1	5	3
			GLE/PIB 12W	96% (258/270)	1% (4/270)	1	3	8
			SOF+DCV 12W	97% (111/115)	1% (1/115)	0	1	3
		IFN- or P/R-exp	GLE/PIB 12W	88% (36/41)	12% (5/41)	1	4	0
			GLE/PIB 16W	92% (12/13)	8% (1/13)	0	1	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (7/7)	0% (0/7)	0	0	0
			GLE/PIB 16W	100% (9/9)	0% (0/9)	0	0	0
		SOF/LDV exp	GLE/PIB 12W	1/1	0/1	0	0	0
	Y	Naïve	GLE/PIB 12W	98% (64/65)	0% (0/65)	0	0	1
			GLE/PIB + RBV 12W	100% (24/24)	0% (0/24)	0	0	0
		IFN- or P/R-exp	GLE/PIB 16W	92% (24/26)	8% (2/26)	1	1	0
			GLE/PIB + RBV 12W	100% (3/3)	0% (0/3)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 16W	96% (24/25)	4% (1/25)	0	1	0
GT4	N	Naïve	GLE/PIB 8W	92% (36/39)	0% (0/39)	0	0	3
			GLE/PIB 12W	100% (71/71)	0% (0/71)	0	0	0
		IFN- or P/R-exp	GLE/PIB 8W	100% (7/7)	0% (0/7)	0	0	0
			GLE/PIB 12W	98% (40/41)	0% (0/41)	0	0	1
	Y	Naïve	GLE/PIB 12W	100% (12/12)	0% (0/12)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (7/7)	0% (0/7)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 12W	1/1	0/1	0	0	0
GT5	N	Naïve	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (22/22)	0% (0/22)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (2/2)	0% (0/2)	0	0	0
GT6	N	Naïve	GLE/PIB 8W	88% (7/8)	0% (0/8)	0	0	1
			GLE/PIB 12W	100% (27/27)	0% (0/27)	0	0	0
		IFN- or P/R-exp	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (4/4)	0% (0/4)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	1/1	0/1	0	0	0

HCV GT1, NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

Considering the regimens proposed for labeling, among noncirrhotic HCV GT1 infected subjects treated with GLE/PIB for 8 weeks, 1/387 (0.3%) subjects experienced virologic failure. The single virologic failure subject experienced on-treatment failure and not relapse and thus a longer treatment duration would not have influenced treatment outcome. Therefore, GLE/PIB for 8 weeks is well supported for noncirrhotic, HCV GT1 infected patients. Among cirrhotic HCV GT1 infected subjects treated with GLE/PIB for 12 weeks, 1/101 (1%) experienced virologic failure (relapse).

The near 100% efficacy of GLE/PIB indicates that natural baseline HCV resistance-associated polymorphism did not significantly influence treatment efficacy for HCV GT1 infected subjects. Considering 9 resistance-associated amino acid positions in NS5A, amino acid polymorphisms were detected at the ≥15% nucleotide sequence level in 21% (78/380) and 32% (147/461) of GT1a- and GT1b-infected subjects, respectively, who had no previous exposure to NS3 protease and NS5A inhibitors. Both virologic failure subjects had GT1a virus with two resistance-associated polymorphisms, although only one polymorphism was detected in one subject at the ≥15% level. Ten subjects with HCV GT1a infection had ≥2 NS5A resistance-associated polymorphisms detected at the ≥15% level, and all 10 achieved SVR12. Both virologic failure subjects also had ≥2 treatment-emergent/enriched NS5A resistance-associated substitutions (RASs) detected at the time of virologic failure, and one subject had a treatment-emergent NS3 A156V substitution.

HCV GT2, NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

Among HCV GT2 infected subjects, virologic failure occurred in 2/197 (1%) noncirrhotic subjects treated with GLE/PIB for 8 weeks. Both failures were due to relapse and occurred in treatment-experienced subjects. One of these subjects had low GLE exposure possibly related to a medical history of gastric bypass. The 2/197 (1%) relapse rate with no evidence of baseline or treatment-emergent resistance in the 2 relapsers (see below) support recommending GLE/PIB for 8 weeks for noncirrhotic, HCV GT2 infected patients. Furthermore, all 35 cirrhotic HCV GT2 infected subjects treated with GLE/PIB for 12 weeks achieved SVR12.

As in HCV GT1 infected subjects, the near 100% efficacy of GLE/PIB for subjects with HCV GT2 infection indicates that natural baseline resistance-associated polymorphisms did not significantly affect treatment outcome. Several HCV GT2 subtypes were represented, with subtypes 2a, 2b and 2c being most common. Both virologic failure subjects had a subtype 2a infection with an NS5A M31 sequence, which is the consensus sequence for this subtype. Neither subject had treatment-emergent or treatment-enriched substitutions at known resistance-associated positions.

HCV GT3, NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

Pooled SVR12 rates for HCV GT3 infected subjects ranged from ~90-100% across various GLE/PIB durations and patient populations. For treatment-naïve, noncirrhotic HCV GT3 infected subjects, pooled SVR12 rates (non-VF-censored) were 97% (177/183) and 99% (258/261) for those who received GLE/PIB for 8 or 12 weeks, respectively, with a 1.9% higher relapse rate (2.7% vs. 0.8%) with the 8-week versus 12-week duration. No treatment-naïve, HCV GT3 infected subjects with cirrhosis who received GLE/PIB for 12 weeks experienced virologic failure (n=64, non-VF-censored).

For treatment-experienced (IFN- or SOF-based), HCV GT3 infected subjects without cirrhosis, relapse rates were 8.3% (4/48) and 4.5% (1/22) with 12- and 16-week GLE/PIB durations, respectively. Similarly, a 4% (2/50) relapse rate was observed for treatment-experienced, HCV GT3 infected subjects with cirrhosis who received GLE/PIB for 16 weeks. Therefore, a conservative 16-week GLE/PIB duration is recommended for treatment-experienced (IFN- or SOF-based), HCV GT3 infected subjects with or without cirrhosis in order to reduce the risk of relapse.

Across all GLE/PIB treatment regimens, 98% of HCV GT3 infected subjects had a subtype 3a infection. Considering the totality of resistance data, there was not a clear association between the detection of NS3 resistance-associated polymorphisms and treatment outcome with GLE/PIB for subjects with GT3 infection. However, baseline and treatment-emergent resistance data indicate that certain NS5A resistance-associated polymorphisms, particularly A30K, may influence GLE/PIB efficacy for GT3. The A30K polymorphism was detected at a ≥15% nucleotide sequence level in 6% of GT3a baseline sequences. Among treatment-naïve, noncirrhotic subjects treated with GLE/PIB for 8 or 12 weeks, 9 subjects experienced virologic failure (excluding 1 suspected reinfection), of whom 5 (56%) had subtype 3a virus with an NS5A A30K polymorphism. Relapse rates for GT3a subjects with the A30K polymorphism were 17.6% (3/17) and 0% (0/13) for subjects who received the 8-week and 12-week durations, respectively. The A30K or Y93H polymorphisms were also enriched among treatment-experienced, HCV GT3 infected subjects who experienced virologic failure with GLE/PIB for 12 or 16 weeks, although insufficient data were available to assess the impact of these polymorphisms specifically on the efficacy of the 16-week duration recommended in the label (n=1 each for A30K or Y93H).

Most GT3 infected subjects who experienced virologic failure with a GLE/PIB regimen had treatment-emergent RASs in NS3 and NS5A. A variety of different NS3 amino acid substitutions emerged, including Y56H/N, Q80K/R, A156G, and Q186L/R. In NS5A, Y93H was the predominant treatment-emergent resistance pathway, occurring in 15/18 (83%) subjects. There was no obvious indication that treatment duration (8-16 weeks) influenced the likelihood of detecting NS3 and NS5A RASs at the time of virologic failure. Therefore, one should expect a high likelihood of treatment-emergent RASs in HCV GT3-infected patients who fail treatment with any GLE/PIB treatment duration ≥8 weeks.

For treatment-naïve, noncirrhotic HCV GT3 infected subjects, the decision to recommend GLE/PIB for 8 weeks versus 12 weeks was a major review issue due to the ~2% higher relapse rate observed with the 8-week duration. Further statistical and virology analyses did not identify a specific subgroup that clearly benefited from a 12-week versus 8-week treatment duration, with the possible exception of subjects with the baseline NS5A A30K polymorphism (see above, based on A30K detection in only 3 relapsers). During the review the sponsor reported SVR12 results from an additional 21 HCV GT3 infected subjects with HIV-1 coinfection who received GLE/PIB for 8 weeks in an ongoing trial (EXPEDITION-2), and all 21 subjects achieved SVR12. The sponsor also reported that 5 HCV GT3 infected subjects who failed treatment with GLE/PIB in Phase 2 trials were successfully re-treated with SOF-based regimens outside of GLE/PIB clinical trials. Following numerous internal discussions, the review team concluded, and this reviewer agrees, that GLE/PIB for 8 weeks for treatment-naïve, noncirrhotic HCV GT3 infected patients is well supported based on the high SVR12 rate and low relapse rate (<3%) observed in clinical trials, with a reasonable sample size (n=186 in NDA who received GLE/PIB for 8 weeks).

HCV GT4, NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

No cases of virologic failure occurred among HCV GT4 infected, noncirrhotic subjects who received GLE/PIB for 8 weeks (n=46) or 12 weeks (n=112), or among cirrhotic subjects who received GLE/PIB for 12 weeks (n=20). Although the number of HCV GT4 infected subjects who received the 8-week duration was relatively limited (n=46), the lack of any relapses with this duration, as well as the consistent efficacy overall for HCV GT4 infected subjects, support the use of GLE/PIB for 8 weeks for noncirrhotic, HCV GT4 infected patients.

Several HCV GT4 subtypes were represented, although subtypes 4a and 4d were predominant, consistent with previous DAA trials. Among HCV GT4 infected subjects enrolled at U.S. study sites, 40/47 (85%) had a subtype 4a infection. Given the 0% virologic failure rate for HCV GT4 infected subjects, there was no evidence that baseline polymorphisms in NS3 or NS5A affected treatment outcome.

HCV GT5 or GT6, NS3/4A PI- and NS5A Inhibitor-Naïve Subjects

Of the 32 HCV GT5 infected subjects who received GLE/PIB for 8 or 12 weeks in clinical trials, no subjects experienced virologic failure. Similarly, none of the 48 HCV GT6 infected subjects who received GLE/PIB for 8 or 12 weeks experienced virologic failure. A key review issue was whether the sponsor had sufficient data to recommend GLE/PIB for the shorter, 8-week duration for noncirrhotic HCV GT5 or GT6 infected patients. Ultimately the review team concluded that, at the time of completion of this review, the sponsor did not have sufficient data to support the 8-week duration for these HCV genotypes.

For GT5, only 2 subjects received GLE/PIB for 8 weeks, and these 2 subjects did not appear to be representative of the broader GT5 population. An NS3 D168E polymorphism was detected in 13/31 (42%) of HCV GT5a infected subjects with available data, but all 13 subjects received GLE/PIB for 12 weeks. Furthermore, both subjects who received the 8-week duration had low baseline HCV RNA levels; these subjects had the second and third lowest HCV RNA levels of all 32 HCV GT5 infected subjects. The other 30 HCV GT5 infected subjects (28 noncirrhotic, 2 with cirrhosis) received GLE/PIB for 12 weeks.

Similarly for GT6, only 9 subjects (censoring 1 subject who failed for non-virologic reasons; later reported during the review to have achieved SVR12) received GLE/PIB for 8 weeks, while the other 38 subjects (31 noncirrhotic, 7 with cirrhosis) received GLE/PIB for 12 weeks. While there were no NS3 polymorphisms of concern as in GT5, GT6 is highly diverse with at least 28 reported subtypes, with amino acid variability at multiple NS5A resistance-associated positions across different GT6 subtypes.

The Division is generally flexible in allowing some extrapolation of results for lower prevalence HCV GTs like GT5 and GT6. However, even with the 12-week duration some extrapolation of available data needs to be considered to support treatment recommendations for GT5- or GT6-infected patients with compensated cirrhosis or prior sofosbuvir- or interferon-based treatment experience. The sponsor has an ongoing trial, M16-126, that will assess the efficacy of the 8-week duration in additional noncirrhotic subjects with HCV GT5 or GT6 infection, which will ideally provide sufficient data to support recommending GLE/PIB for 8 weeks for these populations.

HCV GT1, Treatment-Experienced with NS3/4A PIs and/or NS5A Inhibitors (MAGELLAN-1)

The MAGELLAN-1 trial was a two-part, Phase 2b trial that evaluated GLE/PIB with or without RBV in patients with NS3/4A PI or NS5A inhibitor treatment experience. A total of 141 subjects enrolled across 5 different arms, of whom 137 (97%) had HCV GT1 infection and 4 (3%) had GT4 infection. The review team's analyses focused primarily on GT1 infected subjects, and due to lack of data the review team did not consider a treatment indication for NS3/4A PI- or NS5A inhibitor-experienced subjects with non-GT1 infection. Clinical Virology efficacy and resistance analyses pooled subjects who received GLE/PIB for 12 weeks across Arms A, C and D since the only differences in treatments administered were the DAA formulations used and the slightly lower GLE and PIB dose levels administered in Arm A (n=6, all achieved SVR12). Arm B subjects received GLE/PIB plus RBV for 12 weeks, and Arm E subjects received GLE/PIB for 16 weeks.

Overall SVR12 rates ranged from 86-100% across treatment arms. Virologic failure occurred in 10/107 (9%) and 1/26 (4%) subjects with HCV GT1a or GT1b infection, respectively (non-VF-censored population). All 11 subjects who experienced virologic failure had treatment experience with an NS5A inhibitor-containing regimen. Results on the impact of cirrhosis status on treatment outcome were inconclusive due to limited data, particularly when accounting for GLE/PIB treatment regimen and prior DAA class experience.

Among HCV GT1 infected subjects who were NS3/4A PI-experienced and NS5A inhibitor-naïve, GLE/PIB for 12 weeks resulted in an SVR12 rate of 26/26 (100%, non-VF-censored), with no obvious benefit of dosing

GLE/PIB for >12 weeks or in combination with RBV, supporting the use of GLE/PIB for 12 weeks in this population.

Among HCV GT1 infected, NS5A inhibitor-experienced, NS3/4A PI-naïve subjects, non-VF-censored SVR12 rates were 90% (18/20) and 94% (16/17) for those who received GLE/PIB for 12 or 16 weeks, respectively. Efficacy was lower for those with prior NS5A inhibitor and NS3/4A PI experience, with SVR12 rates of 83% (19/23) and 80% (12/15) with the 12- and 16-week durations, respectively. Although SVR12 rates were similar with the 12- and 16-week durations, all 4 instances of virologic failure with the 16-week duration occurred on-treatment, while all instances of relapse occurred among subjects who received the 12-week duration, providing some evidence that a 16-week duration may reduce the risk of relapse. In general, the numbers of subjects who received GLE/PIB + RBV were insufficient to determine if RBV improves treatment efficacy.

Not surprisingly, prior NS5A inhibitor experience was associated with the presence of baseline NS5A resistance-associated substitutions (RASs) that either existed as natural baseline amino acid polymorphisms or emerged from prior NS5A inhibitor experience. Virologic failure with GLE/PIB treatment was associated with the presence of baseline NS5A RASs, particularly at position Q30 or at multiple NS5A positions, and baseline NS3 D168 RASs were also associated with virologic failure for subjects with prior NS5A inhibitor and NS3/4A PI experience. Most virologic failure subjects had treatment-emergent or evolving RASs in NS3 or NS5A, or in both targets, indicating the potential for further treatment-emergent resistance selection with GLE/PIB failure.

A key review issue related to the MAGELLAN-1 trial was whether the sponsor had sufficient evidence to support a treatment recommendation for HCV GT1 infected, NS5A inhibitor-experienced patients. Ultimately the review team concluded that the data support a treatment recommendation for GLE/PIB for 16 weeks for HCV GT1 infected, NS5A inhibitor-experienced, NS3/4A PI-naïve patients. However, the data were not supportive for a treatment recommendation for GT1 infected patients with experience to both NS5A inhibitors and NS3/4A PIs due to higher rates of treatment failure in this subpopulation.

3. ADMINISTRATIVE

3.1 Reviewer's Signature

_____
Patrick R. Harrington, Ph.D.
Senior Clinical Virology Reviewer, Division of Antiviral Products

3.2 Concurrence

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Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antiviral Products

OND CLINICAL VIROLOGY REVIEW

1. INTRODUCTION AND BACKGROUND

1.1 Important Milestones in Product Development

The original IND 116169 and IND 116170 submissions for glecaprevir (GLE, ABT-493) and pibrentasvir (PIB, ABT-530), respectively, were received on 10/15/2012. The original IND 127416 submission for the co-development of GLE/PIB was received on 10/16/2015. An End-of-Phase 2 meeting was held on 11/19/2015 (written responses only). The GLE/PIB combination was granted FDA Breakthrough Therapy designation on 4/21/2016 for the treatment of hepatitis C virus (HCV) genotype 1 (GT1) infected patients with prior direct-acting antiviral (DAA) treatment experience. A Pre-NDA meeting was held with the sponsor on 11/3/2016.

1.2 Methodology

This section summarizes the key clinical virology technical procedures that were used for Phase 2b/3 clinical trials to support the GLE/PIB NDA. Additional methodologies for other nonclinical and clinical virology analyses are summarized as needed throughout this review.

HCV Genotype/Subtype Determination
Three laboratory assays were used to determine HCV genotypes and subtypes in clinical trials. At Screening, HCV genotype and subtype were determined using the Versant® HCV Genotype Inno-LiPA Assay, Version 2.0 or higher (LiPA; Siemens Healthcare Diagnostics, Tarrytown, NY). If the LiPA assay was unable to genotype (or subtype for GT1) a sample at Screening, its genotype (or genotype and subtype) was to be determined by the central laboratory based on a Sanger population sequencing assay of a 329 nucleotide region of the NS5B gene (Koletzki et al., 2010; Murphy et al., 2007). Subsequent confirmation or identification of HCV genotypes/subtypes was conducted based on phylogenetic analysis of NS3/4A and/or NS5A nucleotide sequences used for resistance analyses of baseline isolates.

For the purposes of analyzing efficacy and resistance data, a "derived" subtype assignment was used as the primary determination of HCV genotype and subtype based on the following priority of results: (1) NS3/4A or NS5A phylogenetic analysis, (2) NS5B phylogenetic analysis, and (3) LiPA assay. In general, available HCV *genotype* (and subtype 1a/1b) results were highly concordant across these different methods used, but differences are noted below where applicable. Of note, it is well established that the LiPA assay does not consistently or accurately determine HCV *subtypes* other than 1a and 1b.

HCV Viral RNA Assessments and Primary Efficacy Analyses
HCV RNA levels in Phase 3 trials were determined using the FDA-approved Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0, which has a reported lower limit of quantification (LLOQ) of 15 IU/mL regardless of HCV genotype. HCV RNA levels in Phase 2b trials M14-867 (SURVEYOR-1) and M14-868 (SURVEYOR-2) were determined using the FDA-approved Roche COBAS® TaqMan® Real-Time RT-PCR Assay v2.0, which has a reported LLOQ of 25 IU/mL regardless of HCV genotype. These analyses were conducted by (b) (4)

For all trials the primary efficacy variable was SVR12, defined as HCV RNA <LLOQ at Post-Treatment Week 12. Backward imputation of SVR12 was applied, when sufficient data were available, for subjects missing data in the SVR12 analysis window. All subjects administered study drug in Phase 3 trials were to be followed for

24 weeks post-treatment, and a final analysis will be performed after all enrolled subjects have completed the Post-Treatment Week 24 visit or prematurely discontinued from the study.

Resistance Assessments

Genotypic resistance analyses to support the GLE/PIB NDA were conducted primarily by using a next generation nucleotide sequencing (NGS) assay. A subset of subjects from Phase 2b trials M14-867 (SURVEYOR-1) and M14-868 (SURVEYOR-2) had samples analyzed by Sanger population nucleotide sequence analyses; other than certain analyses of polymorphism prevalence, these subjects did not contribute to the resistance analyses because they received GLE and/or PIB doses that were below the to-be-marketed dose level, or GLE/PIB + RBV.

For detailed NGS results and details on technical aspects of the NGS analyses, please see the Clinical Virology review by Dr. Eric Donaldson, who independently reviewed the sponsor's NGS analysis methods and raw fastq data. Briefly, HCV RNA was purified from clinical plasma samples and the NS3/4A and NS5A coding regions were RT-PCR-amplified using HCV subtype-specific primer sets (based on preliminary subtype determinations). Only samples with ≥1,000 IU/mL HCV RNA were processed for RT-PCR to reduce the chances of oversampling bias. RT-PCR products were then analyzed by (b) (4) Nucleotide sequence data were analyzed to determine or confirm HCV subtypes based on consensus sequences for each target gene, and amino acid substitutions/polymorphisms in the target genes were reported relative to subtype-specific reference sequences. NGS sensitivity thresholds were originally set at 1% by the contract laboratories; however, based on an unexpectedly high frequency of stop codons and polymorphisms observed the sponsor believed that a large number of sequencing artifacts occurred near the sensitivity cutoff, so the sponsor decided to change the minimum NGS analysis cutoff from 1% to 2%. Most of the key resistance analyses conducted for this review used a 15% NGS sensitivity cutoff, which is comparable to Sanger population nucleotide sequencing and is also more easily applied in a clinical setting. Therefore, assay technical issues occurring near the 1-2% NGS sensitivity level did not significantly impact this review. Additional details about the resistance analysis methods (e.g., amino acid positions considered, subject censoring) are provided in Sections 5 and 6.

1.3 Prior FDA Virology reviews

This is the original NDA submission and initial Clinical Virology review of NDA 209394 for GLE/PIB. Previous Clinical Virology reviews of the individual INDs 116169 and 116170 for GLE and PIB, respectively, as well as the combination product IND 127416, were conducted primarily by Dr. Patrick Harrington, Ph.D.

1.4 Major Virology Issues that Arose During Product Development

In general, there were no major virology issues that arose during the clinical development of the GLE/PIB combination. Impressive efficacy was demonstrated in Phase 2 and Phase 3 trials across various HCV genotypes and patient populations. The relatively small numbers of virologic failure cases across Phase 2/3 trials were usually associated with evidence of baseline or treatment-emergent drug resistance, consistent with previous experience with HCV DAA regimens.

Based on early preliminary results from Phase 2 trial M14-868 (SURVEYOR-2), the IND review team initially was concerned that GLE/PIB may have reduced efficacy for pegylated interferon α and ribavirin (Peg-IFNα/RBV) treatment-experienced patients with HCV GT3 infection. This concern led the sponsor to conduct a

more comprehensive and conservative evaluation of efficacy in patients with HCV GT3 infection, which ultimately contributed to the complexity of Phase 2/3 trials that included this population.

In addition, as robust efficacy results (across HCV genotypes) with 12 week durations of GLE/PIB emerged from Phase 2 trials, the sponsor expanded the clinical evaluation of an 8-week duration of treatment for noncirrhotic subjects, which further contributed to the complexity of the clinical development program as additional treatment arms were incorporated into ongoing Phase 2b trials or new Phase 3 trials.

While impressive efficacy was observed with the 8-week duration in noncirrhotic subjects, unfortunately minimal data with this treatment duration were generated for certain patient populations, particularly HCV GT5 and GT6 infected subjects. The NDA review team ultimately concluded that available data for noncirrhotic patients with HCV GT5 or GT6 infection were insufficient to support an 8-week treatment duration; rather a 12-week treatment duration is better supported for these genotypes and should be recommended in labeling. The concern about limited data with the 8-week treatment duration for HCV GT5 and GT6 was communicated to the sponsor during the Pre-NDA meeting for this application.

The GLE/PIB clinical development program also included patients with prior NS3/4A protease inhibitor (PI) or NS5A inhibitor treatment experience. Preliminary efficacy results in NS3/4A PI or NS5A inhibitor treatment-experienced subjects with HCV GT1 infection were favorable and supported an FDA Breakthrough Therapy designation for this patient population. Additional data included in the NDA provide further evidence of efficacy in this population; however, as discussed in this review GLE/PIB was not optimally effective for patients with prior NS3/4A PI and NS5A inhibitor treatment experience, which appears to be explained at least in part by the presence of virus with resistance-associated amino acid substitutions. Furthermore, the sponsor's proposed labeling included (b) (4) During the Pre-NDA meeting for this application the review team informed the sponsor that that any treatment recommendations (b) (4)

1.5 State of Antivirals Used for the Indication(s) Sought

According to the U.S. Centers for Disease Control and Prevention (CDC), an estimated 2.7-3.9 million people in the U.S. have chronic HCV infection. The virus is transmitted primarily by the use of contaminated needles, but may also be transmitted by exposure to contaminated blood in healthcare settings, by mother-to-child transmission, or less commonly through sexual contact. Prior to the implementation of screening of the blood supply in the 1990s, HCV was commonly transmitted through blood transfusions and organ transplants. Approximately 15-30% of acute HCV infections are resolved without treatment, while the infection becomes chronic in ~70-85% of cases. Most patients with chronic HCV develop chronic liver disease, ~5-25% of patients eventually develop cirrhosis over a period of ~20-30 years, and a subset of these patients will eventually die due to liver cancer or other complications. Chronic HCV infection is the leading indication for liver transplantation in the U.S., and the CDC reported that in 2014 there were approximately 20,000 deaths in the U.S. with HCV as an underlying or contributing cause of death.

Hepatitis C viruses that circulate in the general population are extremely genetically diverse. As of May 2015 there are at least 7 confirmed genotypes and 84 subtypes of HCV, which are assigned based on nucleotide sequence relatedness of complete or near complete coding sequences of HCV genomes (Smith et al., 2014; https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/35/table-1-confirmed-hcv-genotypessubtypes-may-2015). The most common HCV genotype both in the U.S. and worldwide is genotype 1 (GT1), with subtype 1a being relatively more common than subtype 1b in the U.S. HCV GT1 accounts for approximately 75% of U.S.

chronic HCV infections, GT2, GT3 and GT4 each account for approximately 6-11% of U.S. infections, and GT5 or GT6 account for ≤1% of U.S. infections (Gower et al., 2014; Messina et al., 2015). To this reviewer's knowledge only a few isolates of HCV GT7 have been reported, all originating from the Democratic Republic of Congo (Murphy et al., 2015).

The goal of treatment for chronic HCV is to obtain a sustained virologic response (SVR12) defined as plasma HCV RNA below the lower limit of quantification (<LLOQ, based on a sensitive assay) 12 weeks following the end of treatment. The SVR12 endpoint predicts long term durability of virologic response, is generally considered a virologic cure, and numerous studies have demonstrated correlations between SVR and improvements in clinical outcomes (reviewed in FDA HCV DAA draft guidance).

The treatment of chronic HCV infection has evolved dramatically in recent years with the development and approval of HCV direct-acting antivirals (DAAs). Several combination DAA regimens are now approved in the U.S. and provide highly effective, IFN-free treatment options for all major HCV genotypes and most patient populations. Current treatment guidelines generally recommend treatment durations of 12 to 24 weeks depending on patients' HCV genotype, cirrhosis status, and prior treatment history (AASLD/IDSA HCV treatment guidelines).

No DAA regimens are currently FDA-approved for the treatment of patients with prior treatment experience with NS5A inhibitor-containing regimens. Patients who fail DAA regimens that include an HCV NS5A inhibitor frequently have virus with resistance or reduced susceptibility to one or multiple drugs in the NS5A inhibitor class, and therefore may not respond optimally to currently available NS5A inhibitor-containing regimens.

The present NDA, if approved, will provide a highly effective new treatment option for patients with any major HCV genotype (GTs 1-6), and with treatment durations as little as 8 weeks for noncirrhotic patients with HCV GTs 1-4. The NDA also includes data demonstrating the efficacy of GLE/PIB in HCV GT1 infected patients with prior NS3/4A PI or NS5A inhibitor treatment experience, with the latter representing a current unmet medical need.

2. NONCLINICAL VIROLOGY

2.1 Introduction

Section 2 of this review covers the key non-clinical virology characteristics of GLE and PIB, including each drug's mechanism of action, antiviral activity in cell culture, antiviral activity in the presence of serum proteins, combination antiviral activity with other HCV DAAs, cytotoxicity, drug resistance mechanisms, and the potential for cross-resistance with other anti-HCV agents. Most of the key nonclinical virology characteristics for GLE and PIB were described in the Original IND submissions for these DAAs and were assessed using standard HCV laboratory assays; for additional details see the Clinical Virology reviews of Original INDs 116169 and 116170 for GLE and PIB, respectively.

2.2 Glecaprevir (GLE, ABT-493)

Mechanism of Action

GLE is an HCV NS3/4A protease inhibitor. In biochemical assays, GLE inhibited the activity of recombinant NS3/4A protease enzymes from HCV subtypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a, with IC_{50} values of 3.5-11.3 nM. Data from cell culture resistance selection studies also confirm that GLE targets the NS3/4A protease.

Antiviral Activity in Cell Culture

GLE inhibited the replication of stable full-length or chimeric HCV replicons carrying NS3 protease genes from HCV subtypes 1a, 1b, 2a, 3a, 4a and 6a, with EC_{50} values of 0.85-2.8 nM (Table 3; Report R&D/12/716, pg. 27). In separate studies, GLE was shown to have EC_{50} values of 4.6 nM and 1.9 nM against stable GT2b and GT3a replicon cell lines, respectively. Attempts to generate a cell line with a viable replicon containing a GT5a NS3 protease domain were not successful.

Table 3. Anti-HCV activity of GLE against stable full-length or chimeric HCV replicons. Note that the sponsor reported an EC_{50} value of (b) (4) for a GT2a replicon in a separate study (Report R&D/16/0568, pg. 8), (b) (4)

	0% Human Plasma[a]		40% Human Plasma[a]	
HCV Replicon Subtype	N[b]	Mean EC_{50}, nM, ± Std. Dev.	N	Mean EC_{50}, nM, ± Std. Dev.
GT1a	9	0.85 ± 0.15	10	5.3 ± 1.0
GT1b	8	0.94 ± 0.35	8	10 ± 5.0
GT2a[c]	2	2.7 ± 1.1		ND
GT3a	2	1.6 ± 0.49		ND
GT4a	4	2.8 ± 0.41		ND
GT6a	4	0.86 ± 0.11		ND

a. Both the 0% and 40% human plasma assays also contain 5% fetal bovine serum.
b. Number of independent replicates.
c. Studies conducted at SRI.
ND: Not determined.

GLE had consistent, sub-nanomolar activity against transient HCV replicons derived from HCV GT1a (n=11) and GT1b (n=9) clinical isolates, with median EC_{50} values of 0.08 nM and 0.29 nM, respectively (range 0.05-0.68 nM). GLE also inhibited transient HCV replicons derived from non-GT1 clinical isolates with median EC_{50} values of 1.6 nM for GT2a (n=4), 2.2 nM for GT2b (n=4), 2.3 nM for GT3a (n=2), 0.41 nM for GT4a (n=6), 0.17 nM for GT4d (n=3) and 0.12 nM for GT5a (n=1). The GT5a isolate did not include an NS3 D168E polymorphism that is relatively common for this subtype. In the transient replicon inhibition assays, the GLE EC_{50} values against reference GT1a-H77 and GT1b-Con1 strains were 0.16 nM and 0.25 nM, respectively.

GLE had no measurable cell culture antiviral activity (EC_{50} values >22 µM) against human immunodeficiency virus type 1 (HIV-1) or hepatitis B virus (HBV).

Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins

The presence of 40% human serum reduced the anti-HCV activity of GLE by ~6- to 11-fold in HCV GT1a and GT1b replicon assays.

Cytotoxicity/Therapeutic Index

In an HCV GT1a replicon cell line, GLE had a 50% cytotoxicity value (CC_{50}) of 72 µM, reflecting a therapeutic index of approximately 85,000. Similar levels of cytotoxicity were observed for HepG2 cells (human liver cell line) and MT4 cells (human T lymphocyte cell line), with CC_{50} values of 62 µM and 59 µM, respectively.

In biochemical studies, GLE had no measurable activity (IC_{50} values >200 µM) against a panel of human serine and cysteine proteases including chymase, elastase, cathepsin B, chymotrypsin types II and VII, kallikrein and urokinase.

Combination Antiviral Activity in Cell Culture

In cell culture combination studies, GLE had non-antagonistic antiviral activity with other anti-HCV agents including PIB (NS5A inhibitor), SOF (uridine nucleotide analogue NS5B polymerase inhibitor), and ribavirin (RBV). Exposure of HCV GT1a or GT1b replicon cell lines to combinations of GLE and PIB at 10-fold over their respective EC_{50} values resulted in no surviving drug-resistant replicon-containing colonies, indicating that this 2-DAA combination has a higher resistance barrier relative to the individual DAAs.

GLE did not antagonize the cell culture anti-HIV-1 activity of the HIV-1 protease inhibitors darunavir and lopinavir. Similarly, these HIV-1 protease inhibitors did not affect the anti-HCV activity of PIB against a GT1b replicon.

Resistance Development in Cell Culture and Cross-Resistance

Cells harboring stable HCV replicons from HCV subtypes 1a, 1b, 3a, 4a or 6a were grown for ~3 weeks in the presence of G418 and GLE at concentrations that were 10-, 100-, or 500-fold above the EC_{50} value for the respective replicons to select for GLE-resistant replicon cell lines. GLE-selected NS3 amino acid substitutions in the replicon cell lines are summarized Table 4 (Report R&D/12/716, pg. 32). Across all of the selections the most common substitutions observed in individual replicon clones occurred at NS3 amino acid position A156. Position A156 is a known NS3/4A PI resistance-associated position, and the selection of amino acid substitutions at this position as well as others (including Y56 and D/Q168) indicates that GLE has at least partially overlapping resistance mechanisms with other HCV NS3/4A PIs.

Table 4. Selection and genotypic characterization of HCV replicons resistant to GLE.

GT	Fold over EC_{50}					
	10X		100X		500X	
	Predominant Variants (prevalence[a])	% Colony Survival[b]	Predominant Variants (prevalence[a])	% Colony Survival[b]	Predominant Variants (prevalence[a])	% Colony Survival[b]
1a	Q41R[c] (13/28) I170V[c] (10/28)	0.043	A156T (8/17) A156V (3/17) Q89R + A156T (4/17)	0.0029	NV (1/1)	0.0010
1b	A156T (3/25) A156V (7/25) P89L + A156V (6/25)	0.047	A156V (5/25) P89L + A156V (5/25) A156V + D168V (5/25)	0.030	A156V (5/24) P89L + A156V (6/24) A156V + D168V (8/24)	0.026
3a	P89L (2/10) P89S[c] (2/10)	0.10	Y56H+ Q168R (2/3) A156G (1/3)	0.00030	None	0
4a	A156T (23/36) A156V (9/36)	0.0015	A156T (6/9) A156V (1/9)	0.0018	A156T (5/14) A156V (4/14)	0.0009
6a	D168H[c] (13/25) D168V[c] (7/25)	0.018	0	0	0	0

a. Number of times this variant was found out of the total number of variant colonies analyzed.
b. [Number of surviving colonies/ number of replicon cells input for selection] x 100.
c. Variants with the designated mutation alone or with other mutation(s).
NV: Not viable

In another study, GLE resistance selections were similarly conducted for a full-length GT2a JFH-1 replicon and a chimeric GT2b-based replicon. Consistent with results for other HCV genotypes/subtypes, resistance selection in the GT2a- and GT2b-derived replicons resulted in emergent substitutions primarily at NS3 positon A156 (A156T>A156V>A156M), with a few GT2a clones having a combination of emergent G15D+A156T/V substitutions.

Amino acid substitutions selected by GLE in cell culture, or associated with resistance to other NS3/4A PIs, were engineered into HCV replicons to assess their impact on GLE anti-HCV activity. Table 5 (adapted from Report R&D/16/0492, pgs. 130-139) provides examples of GLE phenotypic analysis data for HCV replicons carrying site-directed amino acid substitutions at NS3 resistance-associated positions. A full listing of available site-directed mutant replicon phenotype data is provided in Appendix A.

GLE maintained activity against replicons harboring various single amino acid substitutions at certain NS3 positions commonly associated with resistance to other NS3/4A PIs, including positions V36, Y56, Q80 and R155. However, GLE activity was reduced by amino acid substitutions at NS3 positions A156 and D168 (or Q168 for GT3), in some cases by as much as >1,000-fold, consistent with the emergence of substitutions at these positions in GLE-selected replicons. Certain combinations of amino acid substitutions including these positions further reduced GLE activity. Of note, in subtypes 1a and 1b combinations of substitutions at positions Q/P89 and A156 reduced GLE activity by >1,600-fold. Previous studies have indicated that substitutions at position Q/P89 may serve as compensatory fitness substitutions to enhance HCV replicon replication (Pelosi et al., 2012; Friborg et al., 2013). As presented in Section 5.6, variability at this position was not clinically associated with treatment failure. The NS3 G15D substitution selected in a small number of GLE-exposed GT2a replicons in combination with A156T/V did not appear to have an effect on HCV replicon fitness or resistance (<2-fold) relative to replicons harboring only single A156T/V substitutions.

Late in the review cycle (SDN 31), the sponsor noted briefly that introduction of an NS3 D168E substitution in an HCV GT5a replicon caused a <5-fold reduction in GLE activity; no other details were provided.

Not surprisingly based on its mechanism of action, GLE maintained activity against a panel of HCV replicons with amino acid substitutions associated with resistance to other DAA classes, including NS5A inhibitors, nucleos(t)ide analogue NS5B polymerase inhibitors, and non-nucleoside NS5B polymerase inhibitors.

Table 5. Phenotypic analysis of GLE against HCV replicons carrying site-directed NS3 amino acid substitutions. Replication capacity (RC) is not shown for chimeric replicons. Highlight indicates EC_{50} fold-changes (FC) ≥10 relative to subtype parent replicons (arbitrary cutoff, not linked to clinical success vs. failure). See Appendix A for full listing.

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
1a	Wild Type (H77)	11	0.21 ± 0.08	0.77 ± 0.24	-	100
1a	V36M	8	0.28 ± 0.10	1.1 ± 0.31	1.4	81
1a	T54S	3	0.20 ± 0.06	0.79 ± 0.22	1.0	6.2
1a	V55I	3	0.05 ± 0.01	0.29 ± 0.02	0.2	81
1a	Y56H	9	0.21 ± 0.06	1.9 ± 0.74	1.0	3.5
1a	Q80K	6	0.19 ± 0.05	0.65 ± 0.06	0.9	91
1a	R155K	14	0.11 ± 0.03	0.48 ± 0.15	0.5	31
1a	A156G	3	0.13 ± 0.03	1.1 ± 0.03	0.6	45
1a	A156T	6	286 ± 92.8	1527 ± 380	1361	5.2
1a	D168A	3	0.84 ± 0.45	7.5 ± 6.8	4.0	35
1a	D168E	6	0.27 ± 0.09	1.7 ± 0.83	1.3	34
1a	D168F	3	11.5 ± 0.18	18.9 ± 1.4	55	4.0
1a	D168H	3	0.91 ± 0.21	7.0 ± 1.8	4.3	24
1a	D168N	3	0.08 ± 0.01	0.60 ± 0.04	0.4	28
1a	D168V	4	0.93 ± 0.28	8.8 ± 2.9	4.4	1.5
1a	D168Y	10	8.6 ± 3.5	24.8 ± 10.1	41	3.5

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
1a	E357K	3	0.34 ± 0.00	1.1 ± 0.10	1.6	131
1a	V36M + R155K	3	0.14 ± 0.03	0.60 ± 0.01	0.7	29
1a	Y56H + D168A	3	8.2 ± 1.8	33.3 ± 3.8	39	46
1a	Y56H + D168E	3	9.8 ± 0.72	33.9 ± 9.3	47	4.7
1a	Y56H + D168V	3	8.9 ± 1.2	46.8 ± 18.6	42	15
1a	Y56H + D168Y	3	9.4 ± 3.7	34.8 ± 13.1	45	1.1
1a	Q89R + A156T	3	753 ± 30.9	>10000	3585	1.0
1a	Q89R + A156V	3	2294 ± 241	>10000	10922	2.0
1a	R155T + D168N	3	5.9 ± 0.71	28.5 ± 12.1	28	52
1a	A156G + D168A	3	6.7 ± 2.2	32.6 ± 3.0	32	9.1
1a	D168V + E357K	3	6.4 ± 1.9	18.6 ± 0.74	30	24
1a	V36M + Y56H + D168A	3	33.9 ± 1.3	116 ± 2.4	162	35
1a	V36M + Y56H + D168E	3	26.7 ± 1.6	101 ± 16.7	127	1.6
1b	Wild Type (Con1)	10	0.47 ± 0.13	1.9 ± 0.51	-	100
1b	A156S	3	0.20 ± 0.08	2.0 ± 1.1	0.4	61
1b	A156T	6	301 ± 61.6	1545 ± 508	640	19
1b	A156V	7	839 ± 181	3440 ± 547	1786	9.2
1b	D168A	3	0.69 ± 0.11	1.8 ± 0.05	1.5	69
1b	D168E	6	0.40 ± 0.08	1.8 ± 0.55	0.9	80
1b	D168F	3	2.5 ± 0.08	10.3 ± 0.27	5.3	105
1b	D168H	3	0.68 ± 0.05	3.1 ± 0.21	1.4	108
1b	D168K	3	5.3 ± 0.38	20.6 ± 2.0	11	50
1b	D168T	3	1.3 ± 0.20	5.3 ± 2.0	2.8	129
1b	D168V	6	1.5 ± 0.43	5.9 ± 1.5	3.2	157
1b	D168Y	3	0.99 ± 0.11	4.5 ± 0.18	2.1	70
1b	Y56H + D168A	3	8.0 ± 1.5	32.0 ± 6.1	17	6.0
1b	Y56H + D168V	3	7.2 ± 1.3	32.9 ± 3.4	15	22
1b	Y56H + D168Y	3	35.6 ± 4.6	73.1 ± 7.9	76	8.0
1b	P89L + A156T	3	787 ± 115	2451 ± 327	1674	113
1b	P89L + A156V	3	1994 ± 729	5894 ± 1885	4243	119
1b	A156T + D168V	3	643 ± 70.9	3272 ± 218	1368	21
1b	A156V + D168V	3	2465 ± 1321	6167 ± 649	5244	17
2a	Wild Type (JFH-1)	18	2.5 ± 0.69	6.2 ± 1.4	-	100
2a	G15D	3	1.1 ± 0.10	4.0 ± 0.33	0.5	85
2a	A156T	3	541 ± 76.9	957 ± 184	216	69
2a	A156V	3	2857 ± 235	4203 ± 367	1143	43
2a	D168A	6	4.8 ± 1.3	9.3 ± 2.0	1.9	75
2a	D168E	12	8.1 ± 1.9	16.1 ± 3.1	3.3	116
2a	D168H	3	7.9 ± 0.52	18.5 ± 2.5	3.2	86
2a	D168V	3	4.9 ± 0.79	13.2 ± 0.91	2.0	129
2a	D168Y	3	6.0 ± 1.3	17.7 ± 2.9	2.4	107
2a	G15D + A156T	3	871 ± 93.1	1647 ± 329	348	64
2a	G15D + A156V	3	3470 ± 352	5646 ± 906	1388	49
2b	Wild Type	12	3.1 ± 0.46	8.9 ± 1.4	-	-
2b	A156M	3	3370 ± 292	4347 ± 653	1087	-
2b	A156T	3	460 ± 172	788 ± 165	148	-
2b	A156V	3	4510 ± 1726	4298 ± 297	1455	-
2b	D168A	9	3.9 ± 1.0	10.9 ± 2.7	1.3	-
2b	D168E	6	6.6 ± 1.8	13.4 ± 3.7	2.1	-
2b	D168F	3	12.3 ± 1.9	24.4 ± 4.4	4.0	-
2b	D168H	3	8.5 ± 0.06	20.8 ± 2.6	2.7	-
2b	D168S	3	3.8 ± 0.57	8.4 ± 0.96	1.2	-
2b	D168T	3	17.4 ± 1.7	40.2 ± 5.6	5.6	-
2b	D168V	9	9.1 ± 1.2	23.6 ± 4.4	2.9	-
2b	D168Y	3	6.6 ± 1.1	16.7 ± 1.9	2.1	-
3a	Wild Type	10	0.55 ± 0.17	7.2 ± 2.8	-	-
3a	Q80R	3	11.5 ± 1.6	296 ± 122	21	-
3a	A156G	3	909 ± 349	3245 ± 463	1654	-
3a	S166T	8	2.6 ± 0.58	75.5 ± 40.9	4.7	-
3a	Q168H	3	0.40 ± 0.07	9.0 ± 4.2	0.7	-
3a	Q168L	3	6.9 ± 1.7	211 ± 178	13	-
3a	Q168R	3	30.0 ± 10.4	305 ± 141	54	-
3a	Y56H + Q168R	3	763 ± 363	4259 ± 1280	1387	-

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
3a	Q80R + S166T	3	29.8 ± 2.4	904 ± 180	54	-
4a	Wild Type	4	0.67 ± 0.23	2.8 ± 0.66	-	-
4a	A156T	3	962 ± 374	1511 ± 603	1436	-
4a	A156V	3	2081 ± 817	6561 ± 3150	3106	-
4a	D168H	3	14.6 ± 6.1	29.5 ± 2.9	22	-
4d	Wild Type	3	0.15 ± 0.04	0.88 ± 0.08	-	-
4d	Y56H	3	0.69 ± 0.14	5.8 ± 2.9	4.6	-
4d	D168V	3	0.28 ± 0.12	1.6 ± 0.16	1.9	-
4d	Y56H + D168V	3	8.7 ± 0.56	26.3 ± 3.8	58	-
6a	Wild Type	3	0.15 ± 0.03	1.0 ± 0.57	-	-
6a	D168A	3	12.2 ± 5.8	32.0 ± 1.8	81	-
6a	D168G	3	28.6 ± 6.5	59.2 ± 22.1	191	-
6a	D168H	3	22.0 ± 5.7	77.7 ± 17.0	146	-
6a	D168V	3	5.8 ± 2.2	24.3 ± 0.82	38	-
6a	D168Y	3	16.3 ± 4.0	34.2 ± 4.6	109	-

2.3 Pibrentasvir (PIB, ABT-530)

Mechanism of Action

PIB is an HCV NS5A inhibitor. Evidence supporting this mechanism of action is primarily derived from cell culture resistance selection studies in which substitutions at certain HCV NS5A positions were shown to reduce the anti-HCV activity of PIB. The symmetrical chemical structure and low pM cell culture antiviral activity of PIB are also characteristic of NS5A inhibitors.

Antiviral Activity in Cell Culture

PIB inhibited the replication of stable full-length or chimeric HCV replicons carrying NS5A genes from subtypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a, with EC_{50} values of 1.4-4.3 pM (Table 6; Report R&D/12/717, pg. 25). In a separate study, PIB had an EC_{50} value of 5.0 pM against the stable HCV GT2a JFH-1 replicon cell line.

Table 6. Anti-HCV activity of PIB against stable full-length or chimeric HCV replicons.

	0% Human Plasma[a]		40% Human Plasma[a]	
HCV Replicon	N[b]	Mean EC_{50}, pM, ± Std. Dev.	N	Mean EC_{50}, pM, ± Std. Dev.
GT1a-H77	10	1.8 ± 0.86	8	64 ± 14
GT1b-Con1	12	4.3 ± 1.7	8	200 ± 54
GT2a-JFH-1[c]	2	1.5 ± 0.71		ND
GT2a	5	2.3 ± 0.65		ND
GT2b	5	1.9 ± 0.59		ND
GT3a	5	2.1 ± 0.66		ND
GT4a	5	1.9 ± 0.61		ND
GT5a	5	1.4 ± 0.36		ND
GT6a	5	2.8 ± 0.67		ND

a. Both the 0% and 40% human plasma assays also contained 5% fetal bovine serum.
b. Number of independent replicates.
c. Studies conducted at SRI.
ND: Not determined.

PIB had consistent, sub- to low pM activity against transient HCV replicons derived from HCV GT1-6 clinical isolates, with median EC_{50} values of 0.89 pM for GT1a (n=11), 2.7 pM for GT1b (n=8), 0.93 pM for GT2a (n=6),

1.3 pM for GT2b (n=11), 0.71 pM for GT3a (n=14), 0.5 pM for GT4a (n=8), 1.2 pM for GT4b (n=3), 1.4 pM for GT4d (n=7), 1.1 pM for GT5a (n=1), 0.74 pM for GT6a (n=3), 0.83 pM for GT6e (n=1), and 0.5 pM for GT6p (n=1). PIB had no measurable cell culture antiviral activity against HIV-1 or HBV, with EC_{50} values of >900 nM and >32 µM for HIV-1 and HBV, respectively.

Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins
The presence of 40% human serum reduced the anti-HCV activity of PIB by ~35-47-fold in HCV GT1a and GT1b replicon assays.

Cytotoxicity/Therapeutic Index
In an HCV GT1a replicon cell line, PIB had a CC_{50} value of >32 µM, reflecting a therapeutic index >10,000,000. Similarly low cytotoxicity was observed for HepG2 cells (human liver cell line) and MT4 cells (human T lymphocyte cell line), with CC_{50} values >10 µM.

Combination Antiviral Activity in Cell Culture
In cell culture combination studies, PIB had non-antagonistic antiviral activity with other anti-HCV agents including GLE (NS3/4A PI), SOF (uridine nucleotide analogue NS5B polymerase inhibitor), and RBV. Exposure of HCV GT1a or GT1b replicon cell lines to combinations of PIB and GLE at 10-fold over their respective EC_{50} values resulted in no surviving drug-resistant replicon-containing colonies, indicating that this 2-DAA combination has a higher resistance barrier relative to the individual DAAs.

Resistance Development in Cell Culture and Cross-Resistance
Cells harboring stable HCV replicons from HCV GT1a or GT1b, were grown for ~3 weeks in the presence of G418 and PIB at a concentrations that were 10-, 100-, or 1,000-fold above its EC_{50} value for the respective replicons to select for PIB-resistant replicon cell lines. PIB-selected amino acid substitutions in the replicon cell lines are summarized in Table 7 (Report R&D/12/717, pg. 29). No PIB-resistant GT1b replicons were selected. However, PIB-resistant GT1a replicon clones were selected and primarily carried an NS5A Y93H substitution. A few GT1a PIB-resistant replicon clones had emergent NS5A Q30D, Q30-deletion, Y93N, or H58D + Y93H substitutions. All of these substitutions occurred at known NS5A inhibitor resistance-associated positions, indicating that PIB has at least partially overlapping resistance mechanisms with other NS5A inhibitors.

Table 7. Selection and genotypic characterization of GT1a and GT1b HCV replicons resistant to PIB.

	Fold Over EC_{50}					
	10X		100X		1000X	
GT	Variant (Prevalence)[a]	% Colony Survival[b]	Variant (Prevalence)[a]	% Colony Survival	Variant (Prevalence)[a]	% Colony Survival
1a	Y93H (18/20) Y93N (1/20)	0.0065%	Q30D (1/4) Y93D (1/4) Q30 deletion (1/4) H58D + Y93H (1/4)	0.0002%	NA	0
1b	NA	0	ND		ND	

a. Number of times this variant was found out of the total number of variant colonies analyzed.
b. (Number of surviving colonies divided by number of input cells) x 100.
NA: Not applicable.
ND: Not done.

Similar PIB resistance selection studies were conducted for a panel of HCV replicons harboring NS5A genes from non-GT1 isolates. In general, few drug-resistant clones were obtained for genotypic analysis (Table 8; Report R&D/16/0567, pg. 8). Only a small number of colonies were obtained from GT2a and GT3a-derived replicons exposed to PIB at a concentration 10-fold over its EC_{50} value, and no PIB-resistant replicon clones could be isolated in the presence of PIB at 100-fold over its EC_{50} value. In the PIB-selected GT2a- and GT3a-derived replicons, substitutions emerged again at NS5A positions known to be associated with resistance to NS5A inhibitors.

Table 8. Selection and genotypic characterization of non-GT1-derived HCV replicons resistant to PIB.

Genotype	NS5A Amino Acid Substitutions	Prevalence[a] at 10X EC_{50}	Prevalence[a] at 100X EC_{50}
2a	F28S + M31I	2/3[b]	No colonies
	P29S + K30G	1/3[b]	
2b	-	No viable colonies[c]	No colonies
3a	Y93H	3/3[d]	No colonies
4a	-	No viable colonies[c]	No colonies
5a	-	No colonies	No colonies
6a	-	No colonies	No colonies

a. Number of times this amino acid substitution was found out of the total number of colonies analyzed. Starting cell number was 1 x 10^6 replicon cells for each 10X or 100X selection.

b. Starting cell number for the GT 2a 10X selection was 2 x 10^6 replicon cells. Two cell culture plates were used for the selection with a starting cell number of 1 x 10^6 cells per plate.

c. Cells did not form distinct colonies during selection and were not viable during subsequent passaging.

d. Cells did not form distinct colonies during selection and only three colonies were viable during subsequent passaging.

Amino acid substitutions selected by PIB in cell culture, or associated with resistance to other NS5A inhibitors, were engineered into HCV replicons to assess their impact on GLE anti-HCV activity. Table 9 (adapted from Report R&D/16/0492, pgs. 140-148) provides examples of PIB phenotypic analysis data for HCV replicons carrying site-directed amino acid substitutions at NS5A resistance-associated positions. A full listing of available site-directed mutant replicon phenotype data is provided in Appendix B.

In general, and despite overlapping resistance mechanisms with other NS5A inhibitors, PIB maintained activity against replicons harboring various single amino acid substitutions associated with resistance to NS5A inhibitors. Among all of the site-directed mutant replicons evaluated across all HCV GTs, the only single NS5A amino acid substitutions that reduced PIB activity ≥10-fold (arbitrary cutoff, not linked to clinical success vs. failure) were M28G and Q30D in GT1a, and P29-deletion and P32-deletion in GT1b. Nevertheless, with the exception of the P32-deletion PIB remained active at picomolar levels against these replicons. The presence of two or more NS5A resistance-associated substitutions had a greater impact on PIB activity, indicating that combinations of substitutions act synergistically to confer resistance to PIB. For genotypes 4-6, none of the NS5A amino acid substitutions evaluated, including both single and multiple substitutions, conferred >2-fold resistance to PIB, although a smaller number of NS5A amino acid substitutions were evaluated for these genotypes.

Of note, certain results from the site-directed mutant replicon analyses indicate that HCV replicon drug selection studies may not fully capture the potential for resistance emergence. The limited selection (or lack of selection) of PIB-resistant clones of GT1b and non-GT1-derived HCV replicons noted above indicates that PIB

may have a very high resistance barrier for the HCV genotypes/subtypes evaluated. However, certain NS5A substitutions or deletions engineered into HCV replicons could still confer major reductions in PIB activity. For example, for GT2a-derived replicons the F28S+M31I substitutions emerged in a small number of clones after exposure to PIB at 10-fold over its EC_{50} value, while no GT2a-derived replicons could be recovered when exposed to PIB at 100-fold over its EC_{50} value. These results are not consistent with the finding that this combination of substitutions conferred a >14,000-fold reduction in PIB susceptibility when engineered directly into a GT2a replicon.

Table 9. Phenotypic analysis of PIB against HCV replicons carrying site-directed NS5A amino acid substitutions. Replication capacity (RC) is not shown for chimeric replicons. Highlight indicates EC_{50} fold-changes (FC) ≥10 relative to subtype parent replicons (arbitrary cutoff, not linked to clinical success vs. failure). See Appendix B for full listing. NA, data not available due to low replication capacity.

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC_{50}	RC (%)
1a	Wild-Type (H77)	11	0.72 ± 0.45	1.8 ± 0.65	-	100
1a	K24R	4	0.25 ± 0.04	0.72 ± 0.03	0.3	172
1a	M28G	3	176 ± 21.4	704 ± 281	244	55
1a	M28T	3	1.5 ± 1.1	4.1 ± 3.4	2.0	100
1a	M28V	3	1.3 ± 0.86	3.7 ± 2.1	1.8	87
1a	Q30D	4	67.7 ± 37.0	244 ± 79.6	94	50
1a	Q30E	3	1.7 ± 0.39	5.1 ± 0.71	2.4	70
1a	Q30G	3	0.93 ± 0.24	3.5 ± 1.2	1.3	47
1a	Q30H	3	0.74 ± 0.21	2.4 ± 1.2	1.0	64
1a	Q30K	6	0.69 ± 0.32	2.9 ± 0.91	1.0	36
1a	Q30R	3	1.2 ± 0.62	3.9 ± 2.1	1.7	60
1a	L31M	3	0.76 ± 0.11	1.6 ± 0.34	1.1	141
1a	P32L	3	1.2 ± 0.43	5.1 ± 0.79	1.7	19
1a	H58D	11	0.80 ± 0.17	3.9 ± 0.99	1.1	66
1a	H58P	4	0.46 ± 0.06	1.5 ± 0.20	0.6	129
1a	A92T	3	0.28 ± 0.03	1.6 ± 0.22	0.4	4.1
1a	Y93C	4	1.2 ± 0.57	3.5 ± 2.1	1.7	24
1a	Y93F	3	0.68 ± 0.20	3.0 ± 0.99	0.9	90
1a	Y93H	6	4.8 ± 1.5	22.9 ± 5.6	6.7	18
1a	Y93N	4	5.1 ± 2.1	19.6 ± 4.6	7.0	25
1a	K24R + Q30R	4	0.29 ± 0.06	1.3 ± 0.12	0.4	83
1a	M28G + Q30R	3	15713 ± 5580	113030 ± 34567	21824	66
1a	Q30H + Y93H	3	12.3 ± 1.2	59.8 ± 6.2	17	35
1a	Q30K + H58D	6	170 ± 40.7	1249 ± 283	235	166
1a	Q30K + Y93H	3	1286 ± 353	7584 ± 1641	1786	14
1a	Q30R + L31M	6	2.1 ± 0.79	7.7 ± 2.8	3.0	49
1a	Q30R + H58D	6	91.0 ± 37.0	604 ± 280	126	50
1a	Q30R + Y93H	3	187 ± 110	661 ± 331	260	21
1a	L31M + Y93H	3	54.3 ± 9.3	295 ± 42.0	75	11
1a	L31M + Y93N	3	140 ± 34.2	748 ± 202	195	31
1a	L31V + Y93H	3	67.8 ± 36.1	328 ± 97.5	94	20
1a	H58D + Y93H	4	1612 ± 272	7764 ± 2077	2238	13
1a	Q30H + H58D + Y93H	3	6737 ± 1085	30913 ± 8712	9357	16
1a	Q30R + L31M + H58D	3	1227 ± 277	7298 ± 2203	1704	77
1b	Wild-Type (Con1)	12	1.9 ± 0.80	3.8 ± 1.2	-	100
1b	L28M	3	1.8 ± 0.11	3.5 ± 0.42	1.0	114
1b	P29 deletion	3	19.3 ± 6.8	85.7 ± 17.4	10	5.6
1b	L31M	3	2.9 ± 1.2	5.8 ± 2.2	1.5	119
1b	P32 deletion	?	1968 ± 203	?	1036	?
1b	Y93H	10	1.1 ± 0.27	3.9 ± 1.2	0.6	73
1b	Y93N	3	1.2 ± 0.25	3.7 ± 0.84	0.6	52
1b	Y93S	3	0.74 ± 0.24	3.0 ± 0.29	0.4	23
1b	L28M + R30Q	3	0.79 ± 0.10	1.9 ± 0.24	0.4	28
1b	L28M + P32 deletion	?	12020 ± 2148	?	6326	?
1b	L28M + Y93H	3	2.2 ± 0.23	5.1 ± 0.17	1.2	104
1b	R30Q + Y93H	3	2.3 ± 0.15	4.8 ± 0.40	1.2	60
1b	L31M + Y93H	3	1.3 ± 0.24	4.3 ± 0.41	0.7	11

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC_{50}	RC (%)
1b	L31V + Y93H	3	1.7 ± 0.31	4.5 ± 0.38	0.9	24
2a	Wild Type (JFH-1)	14	0.99 ± 0.36	4.4 ± 1.6	-	-
2a	F28C	3	1.3 ± 0.21	4.5 ± 0.40	1.3	-
2a	F28S	3	1.2 ± 0.17	7.4 ± 0.75	1.2	-
2a	M31I	6	1.2 ± 0.22	5.6 ± 2.9	1.2	-
2a	Y93H	3	NA	NA	-	-
2a	F28S + M31I	3	14303 ± 2722	39977 ± 10415	14448	-
2b	Wild Type	24	1.2 ± 0.39	4.8 ± 2.4	-	-
2b	L31I	3	1.8 ± 0.15	4.9 ± 0.74	1.5	-
2b	L31M	3	1.5 ± 0.33	5.7 ± 2.3	1.2	-
2b	Y93H	4	NA	NA	-	-
2b	L28F + L31I	3	1.3 ± 0.29	6.1 ± 2.4	1.1	-
3a	Wild Type	16	0.65 ± 0.16	1.7 ± 0.27	-	-
3a	M28T	3	1.1 ± 0.02	2.1 ± 0.13	1.7	-
3a	A30K	6	0.71 ± 0.18	2.8 ± 0.44	1.1	-
3a	Y93H	4	1.5 ± 0.19	9.5 ± 2.8	2.3	-
3a	S24F + M28K	3	173 ± 65.1	865 ± 82.2	267	-
3a	S24F + A30K	3	2.1 ± 0.14	4.4 ± 0.03	3.3	-
3a	A30K + L31I	3	2.4 ± 0.03	11.6 ± 2.3	3.6	-
3a	A30K + Y93H	3	45.1 ± 2.3	180 ± 17.4	69	-
3a	L31I + Y93H	3	9.6 ± 1.5	58.3 ± 13.3	15	-
3a	S24F + M28K + A30K	3	8932 ± 1517	38547 ± 3825	13742	-
3a	A30K + L31I + Y93H	3	2770 ± 353	9134 ± 1118	4262	-
4a	Wild Type	14	0.78 ± 0.14	2.0 ± 0.46	-	-
4a	L28I	3	0.80 ± 0.07	2.3 ± 0.38	1.0	-
4a	L28M	3	0.63 ± 0.17	1.8 ± 0.18	0.8	-
4a	L28V	3	0.85 ± 0.23	2.7 ± 1.5	1.1	-
4a	L30H	3	1.1 ± 0.51	2.6 ± 0.52	1.3	-
4a	P58L	3	0.75 ± 0.07	2.5 ± 0.81	1.0	-
4a	Y93H	3	NA	NA	-	-
4a	L28I + P58L	3	1.3 ± 0.11	3.5 ± 1.1	1.7	-
4d	Wild Type	15	1.5 ± 0.55	4.1 ± 1.2	-	-
4d	L28V	3	1.7 ± 0.29	8.2 ± 2.2	1.1	-
4d	M31I	3	2.1 ± 0.20	4.3 ± 0.56	1.4	-
4d	M31L	3	1.4 ± 0.28	4.6 ± 0.60	1.0	-
4d	T58A	3	1.8 ± 0.32	6.3 ± 0.95	1.2	-
4d	T58P	3	1.7 ± 0.31	6.7 ± 1.1	1.1	-
4d	T58S	3	1.5 ± 0.16	4.1 ± 0.32	1.0	-
4d	Y93H	4	NA	NA	-	-
4d	K24Q + T58P	4	2.0 ± 0.20	4.0 ± 1.0	1.3	-
4d	L28V + T58S	3	1.7 ± 0.26	6.9 ± 0.87	1.1	-
4d	K24Q + T58P + Y93H	4	1.5 ± 0.27	6.3 ± 1.5	1.0	-
5a	Wild Type	4	0.93 ± 0.20	4.3 ± 0.97	-	-
5a	L28I	3	0.98 ± 0.14	6.2 ± 2.3	1.1	-
5a	L31F	3	1.9 ± 0.11	10.5 ± 3.7	2.1	-
5a	L31V	3	0.75 ± 0.24	3.3 ± 0.69	0.8	-
6a	Wild Type	4	1.0 ± 0.31	3.3 ± 1.0	-	-
6a	L31V	3	1.0 ± 0.38	3.1 ± 0.67	1.0	-
6a	T58A	3	1.4 ± 0.45	4.5 ± 1.4	1.4	-
6a	T58N	3	1.8 ± 0.71	9.6 ± 3.4	1.8	-

Not surprisingly based on its mechanism of action, PIB maintained activity against a panel of HCV replicons with amino acid substitutions associated with resistance to other DAA classes, including NS3/4A PIs, nucleos(t)ide analogue NS5B polymerase inhibitors, and non-nucleoside NS5B polymerase inhibitors.

3. RELEVANT CLINICAL FINDINGS FROM OTHER REVIEW DISCIPLINES

3.1 Summary of Clinical Efficacy (Statistics Review)

Statistical reviews of GLE/PIB efficacy were conducted by Drs. LaRee Tracy, M.A., Ph.D., and Therri Usher, Ph.D. Nine clinical trials of GLE/PIB that enrolled >2,000 subjects were reviewed.

The 8-week GLE/PIB regimen for HCV GT1 infected, noncirrhotic patients was mainly supported by the Phase 3 ENDURANCE-1 trial in which subjects achieved an SVR12 rate (95% CI [confidence interval]) of 99% (97, 100), demonstrating superiority against a 91% historical control rate. The 8-week regimen in noncirrhotic HCV GT2 or GT4 infected patients is supported by SVR12 rates of 99% (96, 100) and 92% (80, 97), respectively, in the SURVEYOR-2 trial. Evidence supporting GLE/PIB treatment for GT3 infected, noncirrhotic, treatment-naïve subjects largely came from the active-controlled ENDURANCE-3 trial in which SVR12 rates in 8-week and 12-week GLE/PIB arms were 95% (90, 97) and 95% (92, 97), respectively. The 8-week arm in ENDURANCE-3 was non-randomized and therefore comparisons of noninferiority with the active control were considered statistically inappropriate. The 8-week arm in ENDURANCE-3 failed to achieve superiority against a historical control 92% SVR12 rate, although additional supportive data for the 8-week regimen came from SURVEYOR-II with an SVR12 rate of 96% (83, 99). Due to limited data, the 8-week GLE/PIB regimen was not adequately supported for noncirrhotic patients with HCV GT5 or GT6 infection, but available data supported a 12-week regimen for these genotypes.

Evidence supporting a 12-week GLE/PIB regimen for treatment-naïve or treatment-experienced (P/R/S) cirrhotic patients infected with GT 1, 2, 4-6 came from the EXPEDITION-1 trial, which demonstrated SVR12 rates of 98.9%-100%, although the sample sizes were small for cirrhotic subjects with GT5 or GT6 infection (n=2 and 6, respectively). A 12-week GLE/PIB regimen for GT3, treatment-naïve patients with cirrhosis is supported by an SVR12 rate of 98% (92, 100) in SURVEYOR-2. A 16-week GLE/PIB regimen in GT3, treatment-experienced (P/R/S) patients with or without cirrhosis is supported by a pooled SVR12 rate of 95% (87, 98) in SURVEYOR-2.

In the MAGELLAN-1 trial, HCV GT1 infected subjects who were NS3/4A PI-experienced (NS5A inhibitor-naïve) achieved an SVR12 rate of 92% (75, 98) with no cases of virologic failure with a 12-week GLE/PIB treatment duration. HCV GT1 infected subjects who were NS5A inhibitor-experienced (NS3/4A PI-naïve) achieved an SVR12 rate of 94% (73, 99) with a 16-week GLE/PIB duration, with one on-treatment virologic failure. Among HCV GT1 infected subjects with both prior NS3/4A PI- and NS5A inhibitor experience, SVR12 rates (95% CI) were 80% both with 12-week (58, 92) and 16-week (55, 93) GLE/PIB durations; GLE/PIB did not achieve an acceptable threshold of efficacy for this subgroup due to the lower SVR12 rate and higher number of virologic failures. All but 4 subjects in MAGELLAN-1 were infected with HCV GT1; therefore, these data do not support labeling for GT2-6 for NS5A inhibitor- and/or NS3/4A PI-experienced patients.

For a more detailed FDA statistical review of efficacy please see the joint review by Drs. LaRee Tracy, M.A., Ph.D., and Therri Usher, Ph.D.

3.2 Summary of Clinical Safety (Clinical Review)

Reviews of safety data across Phase 2b/3 trials of GLE/PIB were conducted by Drs. Larissa Stabinski, M.D. and Aimee Hodowanec, M.D. Dr. Stabinski conducted safety analyses for 2,265 subjects who received at least one dose of GLE/PIB across 8 Phase 2b/3 trials, excluding subjects with severe renal impairment who enrolled in EXPEDITION-4 (M15-462). Dr. Hodowanec conducted the primary safety analysis of

EXPEDITION-4. According to Dr. Stabinski's review, the safety database was comprehensive and adequate to assess safety of GLE/PIB for the proposed use.

In the pooled safety analysis (excluding EXPEDITION-4), headache (9%), fatigue (8%), nausea (6%), and diarrhea (5%) were the most common adverse drug reactions (ADRs) observed; with the exception of fatigue all of these ADRs occurred at higher rates in the GLE/PIB arm versus placebo arm in the ENDURANCE-2 trial. Most ADRs were grade 1 or 2 and mild or moderate in severity across trials. There was only one serious adverse event, transient ischemic attack, considered by the Investigator as reasonably related to the study drug. The proportion of subjects who permanently discontinued treatment due to adverse reactions was 0.3% (7/2,265). Gastrointestinal disorders were the most common treatment-emergent adverse events in the pooled safety dataset, but were mostly mild or moderate and were seen in the context of a high background rate of gastrointestinal symptoms in the study population. Fluctuations in blood pressure were observed in approximately 1% of subjects for both systolic and diastolic hypertension in both directions and could represent natural variation, adverse drug reactions, or drug-drug interactions. A liver safety evaluation (including a review by an expert hepatic panel) did not identify a significant liver safety signal, although a relatively small proportion of subjects (approximately 13%) had compensated cirrhosis, and GLE/PIB will be contraindicated for patients with Child-Turcotte-Pugh class C liver disease and not recommended for patients with class B liver disease.

In subjects with severe renal impairment in EXPEDITION-4, ADRs observed with GLE/PIB treatment included pruritus (17%), fatigue (12%), nausea (9%), asthenia (7%), and headache (6%). No subjects experienced a serious adverse reaction. The proportion of subjects who permanently discontinued treatment due to adverse reactions was 2%. The rate of serious adverse events was comparable to that observed in previous HCV trials in subjects with advanced renal disease. The high SVR12 rate (98%) and absence of virologic failures in EXPEDITION-4 indicates that the presence of renal disease likely does not impact the efficacy of GLE/PIB.

For more detailed FDA reviews of safety please see the clinical reviews by Drs. Larissa Stabinski, M.D., Aimee Hodowanec, M.D. and Wendy Carter, D.O. (cross-discipline team leader).

4. CLINICAL VIROLOGY REVIEW OF EFFICACY

4.1 Summary of Key Efficacy Trials

The GLE/PIB NDA is supported by efficacy, safety, and resistance data from 9 Phase 2b or Phase 3 trials. Table 10 briefly summarizes the key design aspects of these trials, and additional study design details are included in the following summaries of efficacy for each trial.

Table 10. Overview of key Phase 2b/3 trials of the GLE/PIB regimen. Abbreviations: DCV, daclatasvir; ESRD, end-stage renal disease; P/R, pegylated interferon alfa plus ribavirin; P/R/S, regimens containing any interferon, pegylated interferon, ribavirin, and/or sofosbuvir; SOF, sofosbuvir; TE, treatment-experienced; TN, treatment-naïve;

Trial Number (Trial Name w/ clinicaltrials.gov link)	Clinical Phase	Population	Regimen(s)
M13-590 (ENDURANCE-1)	Phase 3	GT1, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 8W or 12W
M15-464 (ENDURANCE-2)	Phase 3	GT2, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 12W or Placebo
M13-594 (ENDURANCE-3)	Phase 3	GT3, noncirrhotic, TN	GLE/PIB 8W or 12W, DCV+SOF 12W
M13-583 (ENDURANCE-4)	Phase 3	GT4-6, noncirrhotic, TN or TE (P/R/S)	GLE/PIB 12W
M14-172 (EXPEDITION-1)	Phase 3	GT1,2,4-6, compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB 12W
M15-462 (EXPEDITION-4)	Phase 3	GT1-6, renal imp./ESRD, noncirrhotic or compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB 12W
M14-868 (SURVEYOR-2)	Phase 2b	GT2-6, noncirrhotic or compensated cirrhosis, TN or TE (P/R/S)	GLE/PIB ± RBV 8-16W
M14-867 (SURVEYOR-1)	Phase 2b	GT1,4-6, noncirrhotic or compensated cirrhosis, TN or TE (P/R)	GLE/PIB 8 or 12W
M15-410 (MAGELLAN-1)	Phase 2b	GT1,4, noncirrhotic or compensated cirrhosis, NS3/4A PI- or NS5A inhibitor-experienced	GLE/PIB 12W or 16W

The proposed to-be-marketed formulation of GLE/PIB is a fixed-dose combination (FDC) tablet that consists of 100 mg GLE and 40 mg PIB, and 3 tablets should be administered QD for a total GLE/PIB dose of 300 mg/120 mg. In the Phase 3 trials, GLE/PIB was administered QD as three FDC tablets for a total daily GLE/PIB dose of 300 mg/120 mg. In Phase 2b trials GLE and PIB could have been administered as individual DAA formulations or as the FDC formulation, depending on trial and treatment arm, and in some treatment arms doses of GLE and/or PIB were lower than the total proposed to-be-marketed dose(s).

Section 4 of this review summarizes independent efficacy analyses of the 8 trials conducted in subjects without prior NS3/4A PI or NS5A inhibitor treatment experience. Section 5 summarizes pooled resistance analyses conducted for these same 8 trials.

Efficacy and resistance analyses were conducted separately for the MAGELLAN-1 (M15-410) trial, which enrolled subjects with prior NS3/4A PI or NS5A inhibitor treatment experience. Section 6 of this review summarizes the MAGELLAN-1 efficacy and resistance analyses.

4.2 M13-590 (ENDURANCE-1): GT1, Noncirrhotic

Title
M13-590, "A Randomized, Open-Label, Multicenter Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Adults with Chronic Hepatitis C Virus Genotype 1 Infection (ENDURANCE-1)"

Summary of Design and Study Population
Clinical trial M13-590 (ENDURANCE-1) was a Phase 3, randomized, open-label, multicenter study to evaluate GLE/PIB in noncirrhotic, HCV GT1 infected subjects who were either HCV treatment-naïve or treatment-experienced. Allowable treatment experience included IFNα or Peg-IFNα, with or without RBV, or SOF plus RBV with or without Peg-IFNα (abbreviated as "P/R/S"). Subjects with HCV/HIV-1 coinfection were eligible and could be naïve to antiretroviral treatment or on a stable, qualifying HIV-1 antiretroviral treatment regimen (dolutegravir, raltegravir, or rilpivirine, plus a 2-nucleos(t)ide reverse transcriptase inhibitor backbone) for at least 8 weeks prior to Screening. Subjects who previously failed a regimen including an HCV NS3/4A protease inhibitor or NS5A inhibitor were not eligible. A positive test result for HBV surface antigen was also exclusionary.

The study was designed to enroll approximately 620 subjects. A minimum of 270 subjects per arm were to be HCV mono-infected and DAA treatment-naïve. Approximately 20 subjects per arm were to have HCV/HIV-1 coinfection, and up to 20 subjects per arm could have prior treatment experience with a SOF-based regimen. Subjects were randomized 1:1 to receive either 8 weeks (Arm B) or 12 weeks (Arm A) of GLE/PIB. The sponsor's primary efficacy analysis was based on an ITT-PS population (primary subset), which excluded HCV/HIV-1 coinfected subjects and SOF treatment-experienced subjects.

A total of 704 subjects were randomized, of whom 703 received at least 1 dose of study drug. Of these subjects, 300 (43%) had HCV GT1a infection, and 400 (57%) had HCV GT1b infection; the other 3 subjects had a subtype 1g infection (n=1, based on NS5B sequence) or an undetermined HCV GT1 subtype (n=2). A total of 436 (62%) subjects were treatment-naïve, and 267 (38%) were treatment-experienced, of whom only 3 (0.4% overall) had prior SOF experience.

A total of 33 (5%) subjects were HCV/HIV-1 coinfected, all of whom were on antiretroviral therapy. Two subjects (106106 and 251105) in the ADEFFOUT dataset had a result of HIVSTAT=POSITIVE, but were not considered to be HCV/HIV-1 coinfected (according to variable COINFECT), and were not classified as being on antiretroviral therapy (HIVART variable). In response to a request for more information on these subjects (SDN 18), the sponsor indicated that HIVSTAT was based on the central laboratory result of the HIV-1/HIV-2 antibody screen, but the final status of HCV/HIV-1 co-infection (COINFECT) was provided by the Investigator. Subjects 106106 and 251105 had positive HIV-1/HIV-2 antibody screens that were unconfirmed with further testing.

FDA Clinical Virology Analysis of Efficacy

This reviewer's independent analyses of HCV RNA results are summarized in Table 11 and Table 12 (FDA analyses). Impressive efficacy was observed in both treatment arms, with only a single subject experiencing virologic failure: Subject 106104 (IFNα treatment-experienced, HCV GT1a, HCV monoinfected) in the 8-week arm experienced on-treatment virologic breakthrough at Treatment Week 4. A longer treatment duration would not have prevented virologic breakthrough, and no relapses were observed in the trial, indicating that 8 weeks of treatment with GLE/PIB was equally as effective as 12 weeks of treatment. All 33 HCV/HIV-1 coinfected subjects achieved SVR12 (n=15 in 8-week arm, n=18 in 12-week arm). There was no evidence that HCV GT1 subtype, HCV/HIV-1 coinfection status, or HCV treatment history significantly influenced efficacy in this trial.

Table 11. ITT efficacy analysis of clinical trial ENDURANCE-1 (GT1, noncirrhotic).

Regimen	SVR12	On-Tx VF	Relapse	Non-VF[1]
GLE/PIB 8W (Arm B)	**99.1% (348/351)**	0.3% (1/351)	0% (0/351)	0.6% (2/351)
GLE/PIB 12W (Arm A)	**99.7% (351/352)**	0% (0/352)	0% (0/352)	0.3% (1/352)

[1]Non-virologic failure subjects: 104105 (Arm B, missing SVR12 data), 106107 (Arm B, premature discontinuation on Day 2), 133102 (Arm A, missing SVR12 data).

Table 12. SVR12 (ITT) results in key subpopulations in clinical trial ENDURANCE-1 (GT1, noncirrhotic).

	GLE/PIB 8W	GLE/PIB 12W
HIV Coinfected		
N	99.1% (333/336)	99.7% (333/334)
Y	100% (15/15)	100% (18/18)
Tx History		
Naïve	99.1% (217/219)	99.5% (216/217)
IFN- or P/R-experienced	99.2% (130/131)	100% (133/133)
SOF + RBV or P/R	1/1	100% (2/2)
HCV Subtype-Derived		
1b	100% (197/197)	100% (202/202)
1a	98.0% (150/153)	99.3% (148/149)
1	1/1	n/a
1g	n/a	1/1
HCV Subtype-LiPA		
1b	100% (190/190)	100% (196/196)
1a	98.0% (149/152)	99.3% (140/141)
Missing	100% (8/8)	100% (14/14)
1	1/1	1/1
HCV Subtype-LiPA+NS5B		
1b	100% (198/198)	100% (202/202)
1a	98.0% (149/152)	99.3% (147/148)
1	1/1	1/1
1g	n/a	1/1
HCV Subtype-NS3/4A and/or NS5A		
1b	100% (184/184)	100% (200/200)
1a	148/151 (98.0%)	99.3% (143/144)
Missing	15/15 (100%)	100% (8/8)
1	1/1	n/a

HIV-1 RNA Levels in HCV/HIV-1 Coinfected Subjects

As noted above, all 33 subjects with HIV-1 coinfection were on antiretroviral therapy, which consisted of dolutegravir (n=17), raltegravir (n=10) or rilpivirine (n=6) based therapy. HIV-1 RNA levels were assessed for these subjects throughout the Treatment and Post-Treatment periods-assay using the Roche COBAS® Ampliprep/COBAS® TaqMan HIV-1 Test, v2.0 (LLOQ=20 copies/mL). Failure to maintain HIV-1 virologic suppression was defined as HIV-1 RNA ≥200 copies/mL confirmed on 2 consecutive tests at least 2 weeks apart, in a subject compliant with their HIV-1 antiretroviral therapy.

No subjects met the protocol definition of HIV-1 virologic failure. However, 1 subject (M13590-80624-182113; GLE/PIB 8W, achieved SVR12, raltegravir-based ART) had an HIV-1 RNA result of 356 copies/mL at Post-Treatment Week 12. This subject had quantifiable HIV-1 RNA levels at the GLE/PIB end-of-treatment visit (Week 8, 30 copies/mL), Post-Treatment Week 4 (48 copies/mL), and Post-Treatment Week 12 (356 copies/mL). The sponsor did not discuss this subject in the clinical study report so it is not clear if the subject was compliant with antiretroviral therapy or if any additional follow-up HIV-1 RNA results are available, although based on antiretroviral therapy usage it appears that the subject stopped antiretroviral therapy at Day 170 (GLE/PIB Post-Treatment Week ~16).

Four other subjects had transiently quantifiable HIV-1 RNA during the study, either during GLE/PIB treatment or in the post-treatment period. In all 4 cases HIV-1 RNA was <100 copies/mL and returned to <LLOQ at the next immediate visit.

4.3 M15-464 (ENDURANCE-2): GT2, Noncirrhotic

Title

M15-464, "Randomized, Double-Blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Adults with Chronic Hepatitis C Virus Genotype 2 Infection (ENDURANCE-2)"

Summary of Design and Study Population

Clinical trial M15-464 (ENDURANCE-2) was a Phase 3, randomized, double-blind, placebo-controlled multicenter study to evaluate the efficacy and safety of GLE/PIB in noncirrhotic, HCV GT2 infected subjects who were either HCV treatment-naïve or treatment-experienced. Allowable treatment experience included IFNα or Peg-IFNα, with or without RBV, or SOF plus RBV with or without Peg-IFNα. Positive test results for HBV surface antigen or anti-HIV antibody were exclusionary.

The study was designed to enroll approximately 291 to 321 subjects who were to be randomized 2:1 to receive GLE/PIB for 12 weeks (Arm A) or matching placebo for 12 weeks (Arm B). Subjects in Arm B were to receive GLE/PIB for 12 weeks after the placebo dosing period. The sponsor's primary efficacy analysis excluded subjects who were SOF-based treatment-experienced. The primary analysis occurred after all subjects in Arm A completed the Post-Treatment Week 12 Visit or prematurely discontinued the study. This reviewer's analysis focused only on subjects in Arm A.

A total of 304 subjects were randomized, of whom 302 received at least one dose of study drug or placebo. Of these subjects, 202 received study drug in Arm A. Approximately 30% of subjects were treatment-experienced, although only 6 (3%) subjects in Arm A were SOF-experienced.

FDA Clinical Virology Analysis of Efficacy

This reviewer's independent analyses of HCV RNA results are summarized in Table 13 (FDA analysis). All but one subject in the active treatment arm achieved SVR12. The one subject who did not achieve SVR12 failed

for non-virologic reasons (803201, missing SVR12 result). Note that one subject (809203) had possible late relapse based on a single, unconfirmed HCV RNA measurement of 222 IU/mL at Post-Treatment Week 24.

Table 13. ITT efficacy analysis of clinical trial ENDURANCE-2 (GT2, noncirrhotic).

	SVR12	On-Tx VF or Relapse	Non-VF[1]
GLE/PIB 12W (Arm A) -All Subjects	**99.5% (201/202)**	**0% (0/202)**	**0.5% (1/202)**
Tx-Naïve	99.3% (140/141)	0% (0/141)	0.7% (1/141)
IFN- or P/R-experienced	100% (55/55)	0% (0/55)	0% (0/55)
SOF + RBV or + P/R-exp.	100% (6/6)	0% (0/6)	0% (0/6)

[1]Non-VF Subject: 803201 (missing SVR12 result)

Exploratory Analysis of Efficacy According to GT2 Subtype

HCV GT2 is genetically diverse with at least 15 confirmed subtypes (Smith et al., 2014; https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/35/table-1-confirmed-hcv-genotypessubtypes-may-2015.). However, unlike in HCV GT1, major HCV subtypes within HCV GT2 are not reliably identified by commonly used clinical assays, and treatment decisions with currently approved treatments for HCV GT2 do not consider GT2 subtype.

At least 8 different HCV GT2 subtypes, primarily identified by NS3/4A and/or NS5A phylogenetic analysis, were represented in the active treatment arm of ENDURANCE-2 (Table 14, FDA analysis). Of note, 4 subjects identified as being infected with HCV GT2 at baseline by the LiPA assay were subsequently found to have had a GT1b infection based on NS3/4A and/or NS5A phylogenetic analysis. The reason for the discrepant genotype/subtype assay results is unclear but may be explained by the presence of recombinant GT2/GT1 viruses with recombination breakpoints between the targets for the LiPA assay (near the 5' end of the genome) and phylogenetic analyses (3' half of the genome), which has been described previously (Hedskog et al., 2015). Clearly GT2 subtype did not impact treatment efficacy in ENDURANCE-2 as no subject experienced virologic failure. The non-virologic-failure subject, 803201, had a subtype 2b infection. Table 15 (FDA analysis) provides a breakdown of available HCV subtyping assay results according to the three different laboratory methods used.

Table 14. HCV subtypes identified in subjects treated in ENDURANCE-2 Arm A. Subtype determination is derived primarily based on NS3/4A and/or NS5A phylogenetic analysis, with LiPA results used when phylogenetic analysis results were not available.

HCV Subtype-Derived	N	%
1b	4	2.0%
2-unknown	12	5.9%
2a	72	35.6%
2a/2c	3	1.5%
2b	54	26.7%
2c	45	22.3%
2i	6	3.0%
2k	1	0.5%
2l	3	1.5%
2q	1	0.5%
2t	1	0.5%

Table 15. Breakdown of HCV subtypes in ENDURANCE-2 Arm A according to different genotyping/ subtyping methods. N/A, sample not analyzed or genotype/subtype not determined by indicated assay.

HCV Subtype-Derived	LiPA Assay				NS5B Analysis		NS3/4A and/or NS5A Analysis										
	N/A	2	2a/2c	2b	N/A	2i	N/A	1b	2	2a	2b	2c	2i	2k	2l	2q	2t
1b	0	2	2	0	4	0	0	4	0	0	0	0	0	0	0	0	0
2	0	12	0	0	12	0	4	0	8	0	0	0	0	0	0	0	0
2a	0	30	42	0	72	0	0	0	0	72	0	0	0	0	0	0	0
2a/2c	0	0	3	0	3	0	3	0	0	0	0	0	0	0	0	0	0
2b	0	8	0	46	54	0	1	0	0	0	53	0	0	0	0	0	0
2c	0	21	24	0	45	0	0	0	0	0	0	45	0	0	0	0	0
2i	1	1	4	0	5	1	0	0	0	0	0	0	6	0	0	0	0
2k	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
2l	0	3	0	0	3	0	0	0	0	0	0	0	0	0	3	0	0
2q	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
2t	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1

Of note, in HCV GT1 amino acid changes at NS5A position L31 (most commonly L31M) are associated with resistance to several NS5A inhibitors, while a natural methionine at NS5A position 31 (i.e., M31) is common in GT2 and many GT2 subtypes. Despite the frequent occurrence of NS5A M31 in GT2, GLE/PIB clearly had broad efficacy in HCV GT2 infected subjects. More detailed analyses of baseline resistance are described in Section 5.3.

4.4 M13-594 (ENDURANCE-3): GT3, Noncirrhotic, Treatment-naïve

Title
M13-594, "A Randomized, Open-Label, Active-Controlled, Multicenter Study to Compare Efficacy and Safety of ABT-493/ABT-530 to Sofosbuvir Co-Administered with Daclatasvir in Adults with Chronic Hepatitis C Virus Genotype 3 Infection (ENDURANCE-3)"

Summary of Design and Study Population
Clinical trial M13-594 (ENDURANCE-3) was a Phase 3, partially randomized, open-label, active-controlled, multicenter study to compare the efficacy and safety of GLE/PIB to SOF plus daclatasvir (Daklinza™, DCV, NS5A inhibitor) in treatment-naïve, noncirrhotic, HCV GT3 infected subjects. Positive test results for HBV surface antigen or anti-HIV antibody were exclusionary.

The study was designed to enroll approximately 460 subjects. Subjects were assigned to 3 treatment arms:

- Arm A: GLE/PIB for 12 weeks
- Arm B: SOF 400 mg QD plus DCV 60 mg QD for 12 weeks
- Arm C: GLE/PIB for 8 weeks

Subjects were initially randomized in a 2:1 ratio to Arm A or Arm B, with 230 subjects to be randomized to Arm A and 115 subjects to be randomized to Arm B. After enrollment in Arms A and B was complete, 115 subjects were to be assigned (not randomized) to Arm C. Noninferiority of SVR12 rate was assessed by the sponsor for Arm A to Arm B, and Arm C to Arm A.

A total of 506 subjects were enrolled, of whom 505 received at least one dose of study drug. Nearly all subjects (501/505, 99%) had an HCV subtype 3a infection, confirmed or identified based on NS3/4A or NS5A phylogenetic analysis. Two subjects each were infected with subtype 3b or 3i.

FDA Clinical Virology Analysis of Efficacy
This reviewer's independent analysis of HCV RNA results is summarized in Table 16 (FDA analysis). SVR12 rates were 94.9-96.5% across all three arms. Virologic failure rates were modestly numerically higher in the GLE/PIB arms relative to the SOF+DCV arm.

Table 16. SVR12 (ITT) results in clinical trial ENDURANCE-3 (GT3, noncirrhotic, treatment-naïve).

	GLE/PIB 12W (Arm A)	SOF+DCV 12W (Arm B)	GLE/PIB 8W (Arm C)
SVR12	95.3% (222/233)	96.5% (111/115)	94.9% (149/157)
Virologic Failure	1.7% (4/233)	0.9% (1/115)	3.8% (6/157)
On-Tx VF	0.4% (1/233)	0% (0/115)	0.6% (1/157)
Relapse	1.3% (3/233)	0.9% (1/115)	3.2% (5/157)
Non-VF[1]	3.0% (7/233)	2.6% (3/115)	1.3% (2/157)

[1]Non-VF reasons: premature study discontinuation (n=4 in Arm A, n=1 in Arm B) or missing SVR12 data (n=3 in Arm A, n=2 in Arm B, and n=2 in Arm C)

Of the 2 subjects with HCV subtype 3b infection (145301 and 190301), Subject 145301 was in Arm A and experienced virologic relapse, and Subject 190301 was in Arm C and achieved SVR12. Both subjects with HCV subtype 3i infection (103307 and 149302) were in Arm A and achieved SVR12. As noted below, the presence of a baseline NS5A A30K polymorphism was associated with GLE/PIB virologic failure; see Section 5.4 for more details.

4.5 M13-583 (ENDURANCE-4): GT4-6, Noncirrhotic

Title
M13-583, "Single-Arm, Open-Label Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Adults with Chronic Hepatitis C Virus Genotype 4, 5, or 6 Infection (ENDURANCE-4)"

Summary of Design and Study Population
Clinical trial M13-583 (ENDURANCE-4) was a Phase 3, single arm, open-label, multicenter study to evaluate the efficacy and safety of GLE/PIB in HCV GT4-, GT5-, or GT6-infected subjects without cirrhosis, who were either HCV treatment-naïve or treatment-experienced. Allowable treatment experience included IFNα or Peg-IFNα, with or without RBV, or SOF plus RBV with or without Peg-IFNα. Positive test results for HBV surface antigen or anti-HIV antibody were exclusionary. The study was conducted in Belgium, Canada, France, Italy, Portugal, Spain, United Kingdom, and South Africa, with no study sites in the U.S.

The study was designed to enroll approximately 130 subjects who were to receive GLE/PIB for 12 weeks. A total of 121 subjects were enrolled and received study treatment, of whom 76 (62.8%), 26 (21.5%) and 19 (15.7%) had HCV GT4, GT5 or GT6 infection, respectively. Several GT4 and GT6 subtypes were represented, which is discussed in greater detail below. A total of 39 (32%) subjects were treatment-experienced, of whom none were SOF-experienced.

FDA Clinical Virology Analysis of Efficacy
This reviewer's independent analysis of HCV RNA results is summarized in Table 17 (FDA analysis). All but one subject achieved SVR12 with GLE/PIB for 12 weeks, and no subjects experienced virologic failure.

Table 17. SVR12 (ITT) results in clinical trial ENDURANCE-4 (GT4-6, noncirrhotic).

	SVR12	On-Tx VF or Relapse
GLE/PIB 12W -All Subjects	**99.2% (120/121)**	**0% (0/121)[1]**
GT4	98.7% (75/76)	0% (0/76)[1]
GT5	100% (26/26)	0% (0/26)
GT6	100% (19/19)	0% (0/19)

[1]One subject (303402, GT4a) did not achieve SVR12 due to non-VF (early discontinuation).

Table 18 (FDA analysis) provides a breakdown of HCV genotyping/subtyping results according to the assay method used. When multiple assay results were available there was complete concordance of results at the genotype level. Consistent with other DAA development programs, subtypes 4a and 4d were predominant in GT4, only one subtype of GT5 was observed (5a), and multiple GT6 subtypes were observed.

Table 18. HCV genotypes and subtypes represented in clinical trial ENDURANCE-4, according to assay used. Derived subtype is based on the following priority of results: (1) NS3/4A or NS5A phylogenetic analysis, (2) NS5B phylogenetic analysis, and (3) LiPA assay.

HCV GT (LiPA)	HCV Subtype (NS5B)	HCV Subtype (NS3/4A and/or NS5A)	HCV Subtype-Derived	# Subjects (n=121)
4			4	1
4		4a	4a	26
4			4a/4c/4d[1]	1
4		4d	4d	36
4		4f	4f	1
4		4g	4g	1
4			4h[1]	1
4		4k	4k	4
4		4m	4m	1
4		4o	4o	1
	4	4r	4r	1
	4a	4r	4r	1
	4r	4r	4r	1
	5a	5a	5a	3
5		5a	5a	23
	6		6	1
6		6a	6a	5
6			6c-l[1]	6
6		6e	6e	3
	6h		6h	1
6		6p	6p	1
6		6q	6q	1
6		6r	6r	1

[1]Subtype based on LiPA assay, and therefore may not be reliable.

Table 19 (FDA analysis) provides a breakdown of HCV subtypes by study site geographic location. Note that geographic location does not necessarily reflect where subjects initially became infected. As noted above, none of the subjects in this trial were enrolled at U.S. study sites.

Table 19. HCV subtypes (based on derived result) represented in ENDURANCE-4 according to study site geographic location.

HCV Subtype-Derived	COUNTRY	REGION	# Subjects (n=121)
4	United Kingdom	EUROPE	1
4a	Belgium	EUROPE	1
4a	Spain	EUROPE	3
4a	France	EUROPE	5
4a	United Kingdom	EUROPE	6
4a	Italy	EUROPE	4
4a	Portugal	EUROPE	7
4a/4c/4d	Belgium	EUROPE	1
4d	Belgium	EUROPE	1
4d	Spain	EUROPE	13
4d	France	EUROPE	4
4d	United Kingdom	EUROPE	6
4d	Italy	EUROPE	8
4d	Portugal	EUROPE	4
4f	France	EUROPE	1
4g	France	EUROPE	1
4h	United Kingdom	EUROPE	1
4k	Belgium	EUROPE	1
4k	France	EUROPE	2
4k	United Kingdom	EUROPE	1
4m	Canada	NORTH AMERICA	1
4o	Italy	EUROPE	1
4r	Belgium	EUROPE	2
4r	United Kingdom	EUROPE	1
5a	Belgium	EUROPE	8
5a	Canada	NORTH AMERICA	1
5a	France	EUROPE	4
5a	United Kingdom	EUROPE	1
5a	Italy	EUROPE	1
5a	South Africa	REST OF WORLD	11
6	Canada	NORTH AMERICA	1
6a	Canada	NORTH AMERICA	2
6a	France	EUROPE	3
6c-l	Canada	NORTH AMERICA	4
6c-l	France	EUROPE	1
6c-l	United Kingdom	EUROPE	1
6e	Belgium	EUROPE	1
6e	France	EUROPE	2
6h	France	EUROPE	1
6p	Canada	NORTH AMERICA	1
6q	France	EUROPE	1
6r	France	EUROPE	1

4.6 M14-172 (EXPEDITION-1): GTs 1, 2, 4-6; Cirrhotic

Title
M14-172, "A Single Arm, Open-Label Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Adults with Chronic Hepatitis C Virus Genotype 1, 2, 4, 5 or 6 Infection and Compensated Cirrhosis (EXPEDITION-1)"

Summary of Design and Study Population
Clinical trial M14-172 (EXPEDITION-1) was a Phase 3, single-arm, open-label, multicenter study of GLE/PIB in subjects with HCV GT1, GT2, GT4, GT5 or GT6 infection and compensated cirrhosis. Subjects could be treatment-naïve or treatment-experienced. Allowable treatment experience included IFNα or Peg-IFNα, with or without RBV, or SOF plus RBV with or without Peg-IFNα. Positive test results for HBV surface antigen or anti-HIV antibody were exclusionary.

The study was designed to enroll approximately 175 subjects who were to receive GLE/PIB for 12 weeks. A total of 146 subjects were enrolled and received study treatment, of whom 90 (61.6%), 31 (21.2%), 16 (11.0%), 2 (1.4%), and 7 (4.8%) had GT1, GT2, GT4, GT5 or GT6 infection, respectively. More detailed analyses of HCV genotypes/subtypes are provided below. Most subjects (110/146, 75%) were treatment-naïve. Eleven (7.5%) subjects had prior SOF-based treatment experience.

FDA Clinical Virology Analysis of Efficacy
This reviewer's independent analysis of HCV RNA results is summarized in Table 20 (FDA analysis). Overall, SVR12 was achieved in 99.3% (145/146) of subjects, with the one non-SVR12 subject experiencing virologic relapse: Subject 203501, GT1a infection. All prior treatment-naïve subjects achieved SVR12 (110/110, 100%). Among treatment-experienced subjects, 100% (11/11) of prior SOF-based treatment-experienced subjects achieved SVR12 (n=4, 6 and 1 for GT1, GT2 and GT4, respectively), and 96% (24/25) of IFNα-based treatment-experienced subjects achieved SVR12 (n=20, 1, 3 and 1 for GT1, GT2, GT4 and GT6, respectively).

Table 20. SVR12 (ITT) results in clinical trial EXPEDITION-1 (GTs 1, 2, 4-6; cirrhotic). Note that HCV genotypes/subtypes shown are based on the derived genotype/subtype assignment.

	SVR12	**On-Tx VF or Relapse**
GLE/PIB 12W -All Subjects	**99.3% (145/146)**	**0.7% (1/146)[1]**
GT1	98.9% (89/90)	1.1% (1/90)[1]
GT1a	98% (49/50)	2% (1/50)[1]
GT1b	100% (39/39)	0% (0/39)
GT1g	1/1	0/1
GT2	100% (31/31)	0% (0/31)
GT4	100% (16/16)	0% (0/16)
GT5	100% (2/2)	0% (0/2)
GT6	100% (7/7)	0% (0/7)

[1]One subject (203501, GT1a, P/R-exp.) experienced virologic relapse.

Table 21 (FDA analysis) summarizes the HCV subtypes represented in EXPEDITION-1. Of note, 3 subjects determined at Screening by the LiPA assay to be infected with HCV GT2 were subsequently found to have had HCV GT1a (Subjects 604507, 611501) or GT1b (Subject 104501) based on NS3/4A and/or NS5A phylogenetic analysis, similar to the discordant GT1/GT2 assay results observed in ENDURANCE-2. All non-GT1 infected subjects achieved SVR12. The 3 subjects with discordant GT1/GT2 assay results achieved SVR12.

Table 21. HCV genotypes and subtypes represented in clinical trial EXPEDITION-1, according to assay used. Derived subtype is based on the following priority of results: (1) NS3/4A or NS5A phylogenetic analysis, (2) NS5B phylogenetic analysis, and (3) LiPA assay. Highlighted rows indicate discordant HCV genotype determinations based on the LiPA assay and NS3/4A ± NS5A phylogenetic analysis.

HCV GT or GT1 subtype (LiPA)	HCV Subtype (NS5B)	HCV Subtype (NS3/4A and/or NS5A)	HCV Subtype-Derived	# Subjects (n=146)
1a		1a	1a	46
	1a	1a	1a	2
2		1a	1a	2
1b		1b	1b	38
2		1b	1b	1
1b		1g	1g	1
2			2	2
2		2a	2a	2
2		2b	2b	24
2			2b[1]	1
2		2c	2c	2
4			4	1
	4a	4a	4a	1
4		4a	4a	6
4		4d	4d	4
4		4k	4k	1
4		4o	4o	1
4		4q	4q	2
5		5a	5a	2
6		6a	6a	1
6		6e	6e	5
6		6t	6t	1

[1]Subtype based on LiPA assay, and therefore may not be reliable.

4.7 M15-462 (EXPEDITION-4): GT1-6, Renal Impairment

Title
M15-462, "A Single-Arm, Open-Label, Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Renally-Impaired Adults with Chronic Hepatitis C Virus Genotype 1-6 Infection (EXPEDITION-4)"

Summary of Design and Study Population
Clinical trial M15-462 (EXPEDITION-4) was a Phase 3, single arm, open-label, multicenter study to evaluate GLE/PIB for 12 weeks in HCV GT1-6 infected subjects with severe renal impairment or end-stage renal disease (ESRD), including those on dialysis. Subjects could be noncirrhotic or could have compensated cirrhosis. With the exception of subjects with HCV GT3 infection, all subjects could be treatment-naïve or treatment-experienced; only treatment-naïve HCV GT3 infected subjects were eligible. Allowable treatment experience included IFNα or Peg-IFNα, with or without RBV, or SOF plus RBV with or without Peg-IFNα. Positive test results for HBV surface antigen or anti-HIV antibody were exclusionary.

The study was designed to enroll approximately 100 subjects who were to receive GLE/PIB for 12 weeks. A total of 104 subjects were enrolled and received study treatment, of whom 55 (53%), 16 (15%), 11 (11%), 20 (19%), 1 (1%) and 1 (1%) had GT1, GT2, GT3, GT4, GT5 or GT6 infection, respectively. Twenty (19%) subjects had cirrhosis, 60 (58%) were treatment-naïve, and 2 (2%) had prior SOF-based treatment experience.

FDA Clinical Virology Analysis of Efficacy

This reviewer's independent analyses of HCV RNA results are summarized in Table 22 (FDA analysis). The overall SVR12 rate was 98% (102/104), and no subjects experienced virologic failure.

Table 22. SVR12 (ITT) results in clinical trial EXPEDITION-4: GT1-6, renal impairment. Note that HCV genotypes/subtypes shown are based on the derived genotype/subtype assignment. ND, no data

HCV Genotype	SVR12	On-Tx VF or Relapse	Non-VF
GLE/PIB 12W -All Subjects	98.1% (102/104)	0% (0/104)	1.9% (2/104)[1]
GT1	96.3% (53/55)	0% (0/55)	3.6% (2/55)[1]
GT1a	95.8% (23/24)	0% (0/24)	4.2% (1/24)[1]
GT1b	96.6% (28/29)	0% (0/29)	3.4% (1/29)[1]
GT1g	1/1	0/1	0/1
GT1-unknown	1/1	0/1	0/1
GT2	100% (16/16)	0% (0/16)	0% (0/16)
GT3	100% (11/11)	0% (0/11)	0% (0/11)
GT4	100% (20/20)	0% (0/20)	0% (0/20)
GT5	1/1	0/1	0/1
GT6	1/1	0/1	0/1

[1]Two non-VF subjects: Subject 303603 (GT1a, treatment-naïve, cirrhosis), early discontinuation; Subject 101602 (GT1b, treatment-naïve, cirrhosis), missing SVR12 data.

All 44 treatment-experienced subjects, including the two SOF-experienced subjects (both infected with HCV GT1) achieved SVR12. SVR12 results according to cirrhosis status are summarized in Table 23 (FDA analysis).

Table 23. SVR12 (ITT) results in clinical trial EXPEDITION-4: GT1-6, renal impairment, according to cirrhosis status. Note that HCV genotypes shown are based on the derived genotype/subtype assignment.

HCV GT	Cirrhosis?	SVR12	Non-VF
GT1	N	100% (44/44)	0
	Y	82% (9/11)	18% (2/11)
GT2	N	100% (12/12)	0
	Y	100% (4/4)	0
GT3	N	100% (10/10)	0
	Y	1/1	0
GT4	N	100% (16/16)	0
	Y	100% (4/4)	0
GT5	N	1/1	0
GT6	N	1/1	0

Table 24 (FDA analysis) summarizes the HCV subtypes represented in EXPEDITION-4. Similar to other studies, one subject determined at Screening by the LiPA assay to be infected with HCV GT2 was subsequently found to have had HCV GT1a (Subject 905603, achieved SVR12) based on NS3/4A and/or NS5A phylogenetic analysis. As noted above, the two subjects who did not achieve SVR12 failed for non-virologic reasons: one with GT1a infection and the other with GT1b infection.

Table 24. HCV genotypes and subtypes represented in EXPEDITION-4, according to assay used. Derived subtype is based on the following priority of results: (1) NS3/4A or NS5A phylogenetic analysis, (2) NS5B phylogenetic analysis, and (3) LiPA assay. Highlighted row indicates discordant HCV genotype determinations based on the LiPA assay and NS3/4A ± NS5A phylogenetic analysis.

HCV GT or GT1 subtype (LiPA)	HCV Subtype (NS5B)	HCV Subtype (NS3/4A and/or NS5A)	HCV Subtype-Derived	# Subjects (n=104)
1b		1a	1a	22
	1a	1a	1a	1
2		1a	1a	1
1b		1b	1b	26
1b			1b	1
	1b	1b	1b	2
	1g		1g	1
	1		1	1
2		2a	2a	4
2			2b	1
2		2b	2b	4
2		2c	2c	2
2		2i	2i	1
2		2q	2q	1
2		2	2	2
2			2	1
3		3a	3a	11
4		4a	4a	5
4		4c	4c	1
4		4d	4d	2
4		4g	4g	1
4		4k	4k	3
4		4n	4n	1
	4	4o	4o	1
	4	4r	4r	2
	4r	4r	4r	1
4		4r	4r	1
4		4t	4t	1
4			4	1
5		5a	5a	1
6		6e	6e	1

4.8 M14-868 (SURVEYOR-2): Phase 2b, Non-GT1

Title
M14-868, "A Randomized, Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of ABT-493 and ABT-530 with and without RBV in Subjects with Chronic Hepatitis C Virus (HCV) Genotypes 2, 3, 4, 5 or 6 Infection (SURVEYOR-2)"

Summary of Design and Study Population

Clinical trial M14-868 (SURVEYOR-2) was an expanded Phase 2, randomized, open-label, multicenter study to evaluate the efficacy of GLE and PIB, with and without RBV, in subjects with chronic HCV GT2-6 infection. The study consisted of 4 independent parts (Figure 1; CSR pgs. 175, 177, 178 and 180, respectively). The sponsor considered Part 1 and 2 as "supportive/exploratory" and Parts 3 and 4 as "confirmatory/registrational" in terms of supporting the GLE/PIB NDA. In Parts 1 and 2 of the study, GLE and PIB were administered as separate tablet formulations, while the GLE/PIB fixed dose combination was used in Parts 3 and 4. Allowable treatment-experience in Parts 1 and 2 included only Peg-IFNα/RBV. Treatment-experienced subjects in Parts 3 and 4 may have had prior treatment experience with an IFNα-based regimen (IFNα or Peg-IFNα with or without RBV) or with a SOF-based regimen (SOF plus RBV with or without Peg-IFNα). Among HCV GT2 infected subjects in Part 4/Arm S, a minimum of 90 subjects were to be DAA-naïve. Positive test results for HBV surface antigen or anti-HIV antibody were also exclusionary.

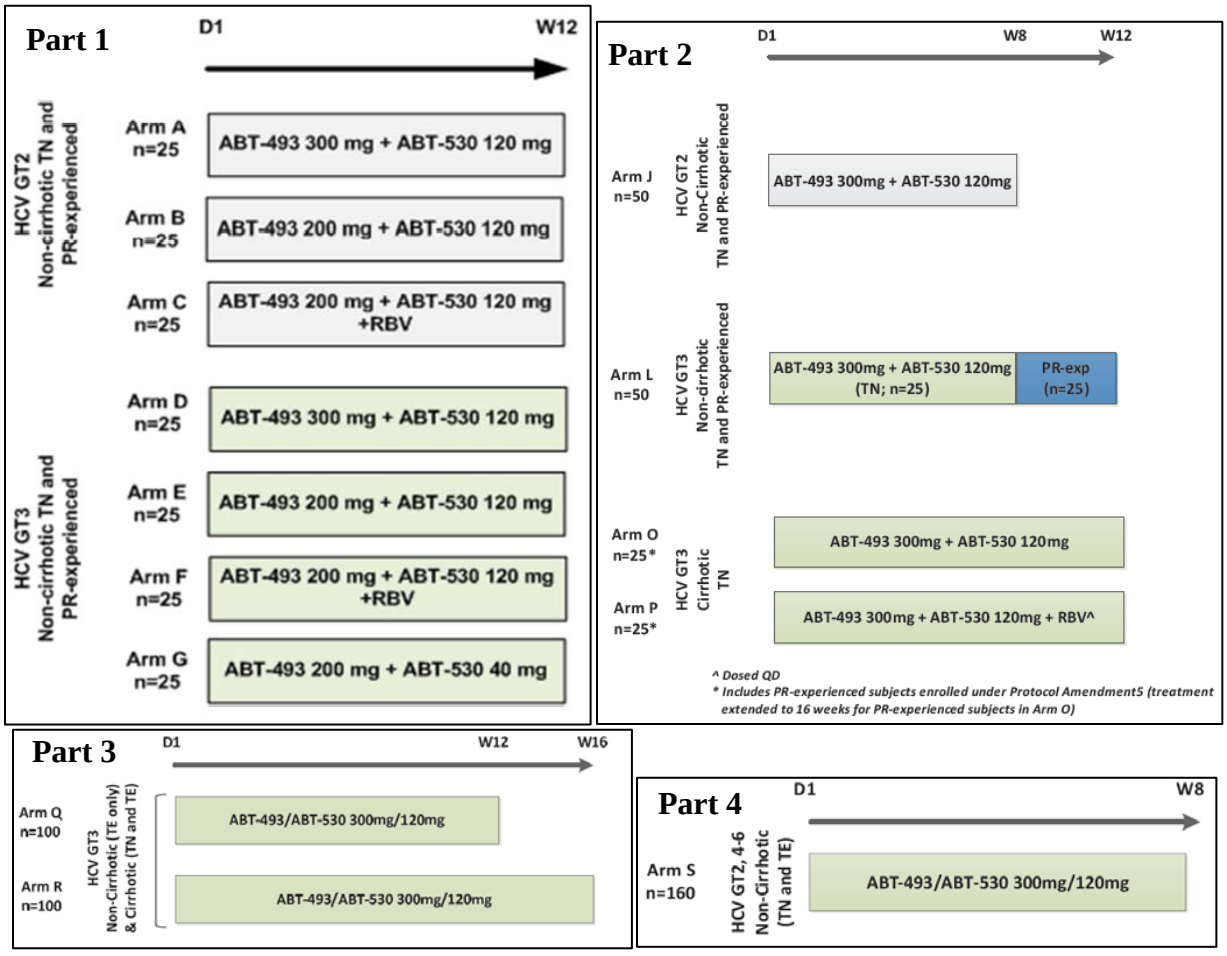


Figure 1. Study design schematic for M14-868 (SURVEYOR-2).

In Part 1, HCV GT2 or GT3 infected subjects without cirrhosis were randomized to the treatment arms shown in Figure 1.

Part 2 was enabled for enrollment based on preliminary efficacy results from Part 1. Several arms in Part 2 were planned but only Arms J, L, O and P were enrolled. Arm L originally planned to evaluate an 8-week duration in noncirrhotic HCV GT3 infected subjects who were either treatment-naïve or treatment-experienced.

Furthermore, HCV GT3 infected subjects with cirrhosis in Arms O and P initially included both treatment-naïve and treatment-experienced subjects. However, based on limited and concerning preliminary data obtained from noncirrhotic, treatment-experienced HCV GT3 infected subjects in Part 1, the Division requested that the sponsor (1) not evaluate an 8-week treatment duration for HCV GT3, treatment-experienced patients in Arm L, and (2) delay investigation of treatment-experienced, HCV GT3 infected subjects with cirrhosis in Arms O and P until supported by favorable efficacy results from noncirrhotic, treatment-experienced, HCV GT3 infected subjects, as well as favorable efficacy results from cirrhotic, treatment-naïve HCV GT3 infected subjects. For more detailed rationale for the requested Part 2 protocol changes, see the Clinical Virology review of IND 116170 SDN 138, and the Division's recommendations communicated to the sponsor on 7/9/2015. Based on the Division's recommendations, Protocol Amendment 6 extended the duration of treatment to 16 weeks for treatment-experienced, HCV GT3 infected subjects with cirrhosis who had already enrolled in Arm O (n=4). Furthermore, the treatment duration for treatment-experienced, noncirrhotic HCV GT3 infected subjects in Arm L was extended to 12 weeks. No changes in protocol treatment were made for treatment-experienced, HCV GT3 infected subjects with cirrhosis who had already enrolled in Arm P (n=3).

In Part 3, following supporting preliminary efficacy results from Part 2, Arms Q and R were opened and enrolled HCV GT3 subjects in the following manner:

- HCV GT3, treatment-naïve, with cirrhosis: assigned to Arm Q (GLE/PIB for 12 weeks) only
- HCV GT3, treatment-experienced, without cirrhosis: randomized 1:1 to Arms Q and R (GLE/PIB for 12 or 16 weeks, respectively)
- HCV GT3, treatment-experienced, with cirrhosis: assigned to Arm R (GLE/PIB for 16 weeks) only

In Part 4, approximately 100 HCV GT2 infected subjects and approximately 60 GT4-6 infected subjects without cirrhosis, with or without prior treatment experience, were enrolled into Arm S to receive GLE/PIB for 8 weeks.

FDA Clinical Virology Analysis of Efficacy

A total of 691 subjects were enrolled and received study treatment in SURVEYOR-2. This reviewer's independent analyses of HCV RNA results are summarized in Table 25 (FDA analysis).

Table 25. SVR12 (ITT) results in clinical trial SURVEYOR-2: Phase 2b, non-GT1 subjects.

Part	Arm	Regimen	HCV GT	Tx History	Cirrhosis?	SVR12	VF	On-Tx VF	Relapse	Non-VF
Part 1	A	GLE/PIB 12W	GT1[1]	Naïve	N	1/1	0/1	0	0	0
			GT2	TN/TE Pooled	N	96% (23/24)	0% (0/24)	0	0	1
				Naïve	N	95% (20/21)	0% (0/21)	0	0	1
				IFN- or P/R-exp	N	100% (3/3)	0% (0/3)	0	0	0
	B	GLE/PIB (200/120) 12W	GT1[1]	Naïve	N	1/1	0/1	0	0	0
			GT2	TN/TE Pooled	N	100% (23/23)	0% (0/23)	0	0	0
				Naïve	N	100% (21/21)	0% (0/21)	0	0	0
				IFN- or P/R-exp	N	100% (2/2)	0% (0/2)	0	0	0
	C	GLE/PIB (200/120) + RBV 12W	GT2	TN/TE Pooled	N	100% (25/25)	0% (0/25)	0	0	0
				Naïve	N	100% (22/22)	0% (0/22)	0	0	0
				IFN- or P/R-exp	N	100% (3/3)	0% (0/3)	0	0	0
	D	GLE/PIB 12W	GT3	Naïve	N	96% (26/27)	0% (0/27)	0	0	1
				IFN- or P/R-exp	N	67% (2/3)	33% (1/3)	0	1	0
	E	GLE/PIB (200/120) 12W	GT3	Naïve	N	100% (27/27)	0% (0/27)	0	0	0
				IFN- or P/R-exp	N	33% (1/3)	67% (2/3)	0	2	0
	F	GLE/PIB (200/120) + RBV 12W	GT3	Naïve	N	93% (26/28)	4% (1/28)	1	0	1
				IFN- or P/R-exp	N	100% (3/3)	0% (0/3)	0	0	0
	G	GLE/PIB (200/40) 12W	GT3	Naïve	N	89% (25/28)	4% (1/28)	0	1	2
				IFN- or P/R-exp	N	0% (0/2)	100% (2/2)	1	1	0
Part 2	J	GLE/PIB 8W	GT2	TN/TE Pooled	N	98% (53/54)	0% (0/54)	0	0	1
				Naïve	N	98% (46/47)	0% (0/47)	0	0	1
				IFN- or P/R-exp	N	100% (7/7)	0% (0/7)	0	0	0
	L	GLE/PIB 8W	GT3	Naïve	N	97% (28/29)	0% (0/29)	0	0	1
		GLE/PIB 12W		IFN- or P/R-exp	N	92% (22/24)	8% (2/24)	1	1	0
	O	GLE/PIB 12W	GT3	Naïve	Y	100% (24/24)	0% (0/24)	0	0	0
		GLE/PIB 16W		IFN- or P/R-exp	Y	75% (3/4)	25% (1/4)	0	1	0
	P	GLE/PIB + RBV 12W	GT3	Naïve	Y	100% (24/24)	0% (0/24)	0	0	0
				IFN- or P/R-exp	Y	100% (3/3)	0% (0/3)	0	0	0
Part 3	Q	GLE/PIB 12W	GT3	Naïve	Y	98% (39/40)	0% (0/40)	0	0	1
				TE Pooled	N	91% (20/22)	9% (2/22)	0	2	0
				IFN- or P/R-exp	N	86% (12/14)	14% (2/14)	0	2	0
				SOF + RBV or P/R exp	N	100% (7/7)	0% (0/7)	0	0	0
				SOF/LDV exp[2]	N	1/1	0/1	0	0	0
	R	GLE/PIB 16W	GT3	Pooled TE	N	95% (21/22)	5% (1/22)	0	1	0
				Pooled TE	Y	96% (45/47)	4% (2/47)	1	1	0
				IFN- or P/R-exp	N	92% (12/13)	8% (1/13)	0	1	0
					Y	95% (21/22)	5% (1/22)	1	0	0
				SOF + RBV or P/R exp	N	100% (9/9)	0% (0/9)	0	0	0
					Y	96% (24/25)	4% (1/25)	0	1	0
Part 4	S	GLE/PIB 8W	GT1[1]	IFN- or P/R-exp	N	100% (2/2)	0% (0/2)	0	0	0
			GT2	TN/TE Pooled	N	98% (140/143)	1% (2/143)	0	2	1
				Naïve	N	99% (126/127)	0% (0/127)	0	0	1
				IFN- or P/R-exp	N	90% (9/10)	10% (1/10)	0	1	0
				SOF + RBV or P/R exp	N	83% (5/6)	17% (1/6)	0	1	0
			GT4	TN/TE Pooled	N	93% (43/46)	0% (0/46)	0	0	3
				Naïve	N	92% (36/39)	0% (0/39)	0	0	3
				IFN- or P/R-exp	N	100% (7/7)	0% (0/7)	0	0	0
			GT5	Naïve	N	100% (2/2)	0% (0/2)	0	0	0
			GT6	TN/TE Pooled	N	90% (9/10)	0% (0/10)	0	0	1
				Naïve	N	88% (7/8)	0% (0/8)	0	0	1
				IFN- or P/R-exp	N	100% (2/2)	0% (0/2)	0	0	0

[1]Four subjects identified with HCV GT2 infection at Screening (LiPA assay) were subsequently found to have had HCV GT1 infection (2 GT1a, 2 GT1b) based on NS3/4A and/or NS5A phylogenetic analysis.
[2]One subject (Subject 1351, GT3) with prior ledipasvir/sofosbuvir experience was enrolled in error, achieved SVR12.

Considering the key registrational part for HCV GT3 infected subjects (Part 3), GLE/PIB for 12 weeks was highly effective in treatment-naïve, HCV GT3 infected subjects with cirrhosis, with a 98% (39/40) SVR12 rate and a 0% (0/40) virologic failure rate (Arm Q). Also in Arm Q, among treatment-experienced, HCV GT3 infected subjects without cirrhosis, 9% (2/22) experienced virologic failure, both of which were relapses. In Arm R in which treatment-experienced, HCV GT3 infected subjects received GLE/PIB for 16 weeks, 5% (1/22) of subjects without cirrhosis experienced virologic failure (relapse), while 4% (2/47) of subjects with cirrhosis experienced virologic failure (1 relapse, 1 on-treatment failure).

In Part 4 (Arm S) in which noncirrhotic subjects with HCV GT2, 4, 5 or 6 received GLE/PIB for 8 weeks, high SVR12 rates and low relapse rates were observed across all HCV genotypes. However, the numbers of subjects with HCV GT5 (n=2) or GT6 (n=10) were small. Furthermore, most subjects were treatment-naïve. Among HCV GT2 infected subjects with prior treatment experience who received GLE/PIB for 8 weeks in Arm J or S, 2/23 (9%) experienced virologic relapse. Of note, according to the SURVEYOR-2 study report, one of the GT2 GLE/PIB 8W virologic relapsers (Subject 2917, SOF-experienced) had GLE exposures that were >75% lower than other subjects in the same treatment arm, while PIB exposures were comparable to other subjects; this subject had a medical history of gastric bypass.

In Part 3, 97% (127/131) of subjects had subtype 3a infection, while the remaining subjects had subtype 3b (n=3) or 3g (n=1) infection. The 5 subjects who experienced virologic failure all had a subtype 3a infection.

HCV subtypes represented in Part 4 (Arm S, GLE/PIB 8W) are summarized in Table 26 (FDA analysis), and efficacy results in Part 4 according to HCV subtype-derived are summarized in Table 27 (FDA analysis). The only 2 virologic relapsers in Arm S had a subtype 2a infection, reflecting a 10% (2/21) virologic relapse rate for this subtype.

Table 26. HCV genotypes and subtypes represented in SURVEYOR-2 Part 4 (Arm S), according to assay used. Derived subtype is based on the following priority of results: (1) NS3/4A or NS5A phylogenetic analysis, (2) NS5B phylogenetic analysis, and (3) LiPA assay. Highlighted rows indicate discordant HCV genotype determinations based on the LiPA assay and NS3/4A ± NS5A phylogenetic analysis.

HCV GT (LiPA)	HCV Subtype (NS5B)	HCV Subtype (NS3/4A and/or NS5A)	HCV Subtype-Derived	# Subjects
2		1a	1a	1
2		1b	1b	1
2		2a	2a	19
	2a	2a	2a	2
2			2a/c[1]	5
2		2b	2b	103
2			2b[1]	4
2		2c	2c	3
2		2q	2q	1
2		2	2	1
2			2	5
4		4a	4a	26
4			4a/c/d[1]	3
4		4d	4d	8
4		4f	4f	1
4		4k	4k	1
	4m	4m	4m	1
4		4v	4v	3
4		4v/4q	4v/4q	1
4			4	2
5		5a	5a	1
5			5a	1

HCV GT (LiPA)	HCV Subtype (NS5B)	HCV Subtype (NS3/4A and/or NS5A)	HCV Subtype-Derived	# Subjects
6		6a	6a	3
6			6a/b[1]	3
6		6e	6e	2
	6e	6e	6e	1
	6l	6l	6l	1

[1]Subtype based on LiPA assay, and therefore may not be reliable.

Table 27. SURVEYOR-2 Part 4/Arm S (GLE/PIB for 8 weeks) efficacy according to HCV subtype-derived.

HCV Subtype	SVR12 (ITT)	Relapse	Non-VF
1a	1/1	0	0
1b	1/1	0	0
2a	90% (19/21)	2	0
2a/c	100% (5/5)	0	0
2b	99% (106/107)	0	1
2c	100% (3/3)	0	0
2q	1/1	0	0
2	100% (6/6)	0	0
4a	96% (25/26)	0	1
4a/c/d	100% (3/3)	0	0
4d	88% (7/8)	0	1
4f	1/1	0	0
4k	1/1	0	0
4m	1/1	0	0
4v	100% (3/3)	0	0
4v/4q	0/1	0	1
4	100% (2/2)	0	0
5a	100% (2/2)	0	0
6a	100% (3/3)	0	0
6a/b	67% (2/3)	0	1
6e	100% (3/3)	0	0
6l	1/1	0	0

Efficacy results from SURVEYOR-2 from Arms in which GLE and PIB were administered at to-be-marketed dose levels were considered in the context of the pooled efficacy and resistance analyses shown in Sections 4.10 and 5, respectively.

4.9 M14-867 (SURVEYOR-1): Phase 2b, GTs1, 4-6

Title
M14-867, “An Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of ABT-493 and ABT-530 with and without Ribavirin in Subjects with Chronic Hepatitis C Virus (HCV) Genotype 1, 4, 5, and 6 Infection (SURVEYOR-1)”

Summary of Design and Study Population
Clinical trial SURVEYOR-1 was a Phase 2, open-label, 2-part study of GLE/PIB in subjects with HCV GT1, GT4, GT5 or GT6 infection. The study design is illustrated in Figure 2 (Clinical Study Report, pg. 65). Both treatment-naïve and P/R treatment-experienced subjects were eligible for all arms. The individual DAA

formulations were administered in all arms. Any prior DAA experience was exclusionary. Positive test results for HBV surface antigen or anti-HIV antibody were also exclusionary.

In Part 1, approximately 80 noncirrhotic subjects with HCV GT1 infection were enrolled into Arm A or Arm B to receive GLE + PIB at two different dose levels for 12 weeks. The regimens in both arms did not include the complete to-be-marketed dose levels of GLE and PIB (lower 200 mg dose for GLE in both arms and lower 40 mg dose of PIB in Arm B).

Part 2 was initiated based on preliminary efficacy and safety results from Part 1. Although several arms in Part 2 were planned, only 3 were enrolled (Figure 2; Clinical Study Report, pg. 65). Arm F enrolled HCV GT1 infected subjects with compensated cirrhosis to receive GLE (200 mg QD, not to-be-marketed dose) + PIB (120 mg QD) for 12 weeks. Arms I and K evaluated to-be-marketed dose levels of both GLE (300 mg QD) and PIB (120 mg QD). Arm I enrolled noncirrhotic subjects with HCV GT4, GT5 or GT6 to receive GLE + PIB for 12 weeks, while Arm K evaluated GLE + PIB for 8 weeks in noncirrhotic subjects with HCV GT1 infection.

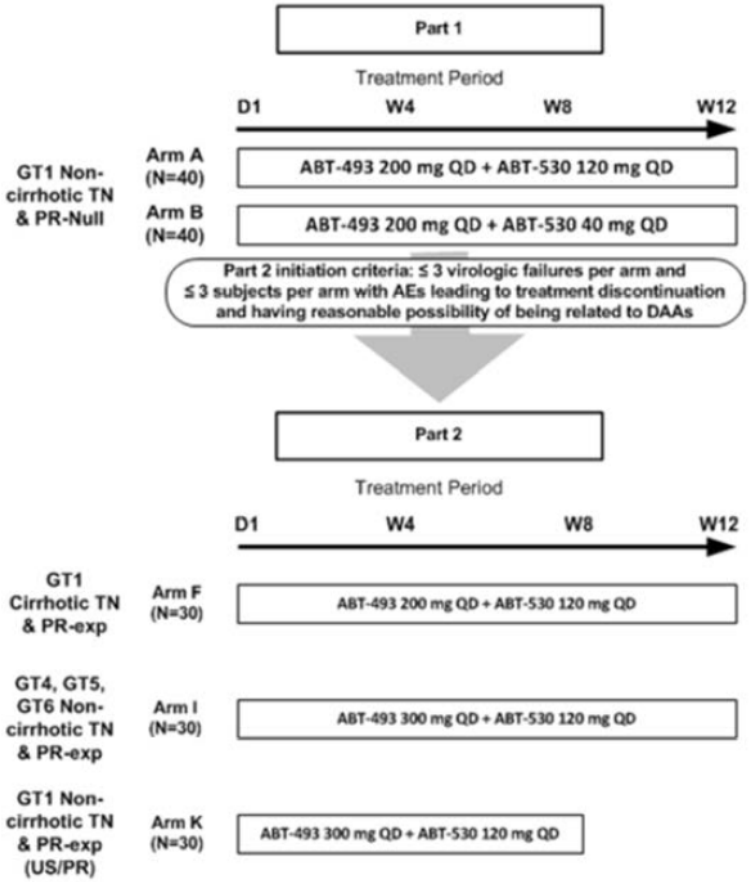


Figure 2. SUVERYOR-I study design schematic.

FDA Clinical Virology Analysis of Efficacy

A total of 174 subjects were enrolled and received study treatment. This reviewer's independent analyses of HCV RNA results are summarized in Table 28 (FDA analysis). Impressive efficacy results were observed for this early Phase 2 trial, with SVR12 rates of 96-100% across all arms. Efficacy results from the SURVEYOR-1 arms in which GLE and PIB were administered at to-be-marketed dose levels (Arms I and K only) were

considered in the context of the pooled efficacy and resistance analyses shown in Sections 4.10 and 5, respectively.

Table 28. SVR12 (ITT) results in clinical trial SURVEYOR-1: Phase 2, GT1 and GT4-6 subjects.

Part	Arm	Regimen	HCV GT	Tx History	Cirrhosis?	SVR12	Virologic Failure	Relapse	Non-VF
Part 1	A	GLE/PIB (200/120) 12W	1	TN/TE Pooled	N	100% (40/40)	0% (0/40)	0	0
				Naïve	N	100% (25/25)	0% (0/25)	0	0
				IFN- or P/R-exp	N	100% (15/15)	0% (0/15)	0	0
			4[1]	Naïve	N	100% (2/2)	0% (0/2)	0	0
	B	GLE/PIB (200/40) 12W	1	TN/TE Pooled	N (incl. 1Y)	97% (38/39)	3% (1/39)	1	0
				Naïve	N	96% (23/24)	4% (1/24)	1	0
					Y[2]	1/1	0/1	0	0
				IFN- or P/R-exp	N	100% (14/14)	0% (0/14)	0	0
Part 2	F	GLE/PIB (200/120) 12W	1	Naïve	N[2]	1/1	0/1	0	0
				TN/TE Pooled	Y	96% (25/26)	4% (1/26)	1	0
				Naïve	Y	95% (19/20)	5% (1/20)	1	0
				IFN- or P/R-exp	Y	100% (6/6)	0% (0/6)	0	0
	I	GLE/PIB 12W	4	TN/TE Pooled	N	100% (20/20)	0% (0/20)	0	0
				Naïve	N	100% (16/16)	0% (0/16)	0	0
				IFN- or P/R-exp	N	100% (4/4)	0% (0/4)	0	0
			5	Naïve	N	1/1	0/1	0	0
			6	TN/TE Pooled	N	100% (11/11)	0% (0/11)	0	0
				Naïve	N	100% (10/10)	0% (0/10)	0	0
				IFN- or P/R-exp	N	1/1	0/1	0	0
	K	GLE/PIB 8W	1	TN/TE Pooled	N	97% (33/34)	0% (0/34)	0	1
				Naïve	N	97% (28/29)	0% (0/29)	0	1
				IFN- or P/R-exp	N	100% (5/5)	0% (0/5)	0	0

[1]Subjects 3311 and 3312, both with GT4 infection, were randomized to Arm I but received the doses in Arm A, which is why they are listed in Arm A.
[2]Two subjects with conflicting cirrhosis results. Subject 2303 (Arm B) had conflicting cirrhosis testing results pre-treatment, but FibroScan done after Post-Treatment Week 24 showed no cirrhosis (shown as having cirrhosis in dataset). Subject 3411 (Arm F) was screened and randomized as having cirrhosis but was found at Week 12 not to have cirrhosis (shown as noncirrhotic in dataset).

4.10 Pooled SVR12 and Virologic Failure Rates: NS3/4A PI-naïve/NS5A Inhibitor-naïve subjects

Table 29 (FDA analysis) provides a pooled summary of efficacy and virologic failure results for 2,398 subjects (NS3/4A PI-naïve and NS5A inhibitor-naïve) who received either (a) GLE and PIB at to-be-marketed dose levels across Phase 2 and Phase 3 trials (n=2,283) or (b) SOF + DCV in the active control arm of M13-594 (ENDURANCE-3) (n=115). Appendix C summarizes these data according to specific trial.

Table 29. Pooled SVR12 and virologic failure results for NS3/4A PI-naïve and NS5A inhibitor-naïve subjects (i.e., excluding MAGELLAN-1/M15-410) who received GLE/PIB to-be-marketed dose levels in Phase 2b/3 trials. Highlighted rows show data for regimens initially proposed by the sponsor for the label.

HCV GT	Cirrhosis	Tx History	Regimen	SVR12	VF Rate	On-Tx VF	Relapse	Non-VF
GT1	N	Naïve	GLE/PIB 8W	99% (245/248)	0% (0/248)	0	0	3
			GLE/PIB 12W	99.6% (241/242)	0% (0/242)	0	0	1
		IFN- or P/R-exp	GLE/PIB 8W	99% (137/138)	1% (1/138)	1	0	0
			GLE/PIB 12W	100% (155/155)	0% (0/155)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 8W	1/1	0/1	0	0	0
			GLE/PIB 12W	100% (4/4)	0% (0/4)	0	0	0
	Y	Naïve	GLE/PIB 12W	97% (69/71)	0% (0/71)	0	0	2
		IFN- or P/R-exp	GLE/PIB 12W	96% (24/25)	4% (1/25)	0	1	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (5/5)	0% (0/5)	0	0	0
GT2	N	Naïve	GLE/PIB 8W	99% (172/174)	0% (0/174)	0	0	2
			GLE/PIB 12W	99% (167/169)	0% (0/169)	0	0	2
		IFN- or P/R-exp	GLE/PIB 8W	94% (16/17)	6% (1/17)	0	1	0
			GLE/PIB 12W	100% (60/60)	0% (0/60)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 8W	83% (5/6)	17% (1/6)	0	1	0
			GLE/PIB 12W	100% (5/5)	0% (0/5)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (26/26)	0% (0/26)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (3/3)	0% (0/3)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
GT3	N	Naïve	GLE/PIB 8W	95% (177/186)	3% (6/186)	1	5	3
			GLE/PIB 12W	96% (258/270)	1% (4/270)	1	3	8
			SOF+DCV 12W	97% (111/115)	1% (1/115)	0	1	3
		IFN- or P/R-exp	GLE/PIB 12W	88% (36/41)	12% (5/41)	1	4	0
			GLE/PIB 16W	92% (12/13)	8% (1/13)	0	1	0
		SOF + RBV or P/R exp	GLE/PIB 12W	100% (7/7)	0% (0/7)	0	0	0
			GLE/PIB 16W	100% (9/9)	0% (0/9)	0	0	0
		SOF/LDV exp	GLE/PIB 12W	1/1	0/1	0	0	0
	Y	Naïve	GLE/PIB 12W	98% (64/65)	0% (0/65)	0	0	1
			GLE/PIB + RBV 12W	100% (24/24)	0% (0/24)	0	0	0
		IFN- or P/R-exp	GLE/PIB 16W	92% (24/26)	8% (2/26)	1	1	0
			GLE/PIB + RBV 12W	100% (3/3)	0% (0/3)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 16W	96% (24/25)	4% (1/25)	0	1	0
GT4	N	Naïve	GLE/PIB 8W	92% (36/39)	0% (0/39)	0	0	3
			GLE/PIB 12W	100% (71/71)	0% (0/71)	0	0	0
		IFN- or P/R-exp	GLE/PIB 8W	100% (7/7)	0% (0/7)	0	0	0
			GLE/PIB 12W	98% (40/41)	0% (0/41)	0	0	1
	Y	Naïve	GLE/PIB 12W	100% (12/12)	0% (0/12)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (7/7)	0% (0/7)	0	0	0
		SOF + RBV or P/R exp	GLE/PIB 12W	1/1	0/1	0	0	0
GT5	N	Naïve	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (22/22)	0% (0/22)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (2/2)	0% (0/2)	0	0	0
GT6	N	Naïve	GLE/PIB 8W	88% (7/8)	0% (0/8)	0	0	1
			GLE/PIB 12W	100% (27/27)	0% (0/27)	0	0	0
		IFN- or P/R-exp	GLE/PIB 8W	100% (2/2)	0% (0/2)	0	0	0
			GLE/PIB 12W	100% (4/4)	0% (0/4)	0	0	0
	Y	Naïve	GLE/PIB 12W	100% (6/6)	0% (0/6)	0	0	0
		IFN- or P/R-exp	GLE/PIB 12W	1/1	0/1	0	0	0

4.11 Durability of SVR12

SVR12/SVR24 Concordance

All 9 Phase 2/3 trial protocols for GLE/PIB included planned follow-up visits through Post-Treatment Week 24 to confirm durability of SVR12 through at least Post-Treatment Week 24 (i.e., SVR24); however, only limited HCV RNA data beyond Post-Treatment Week 12 were reported in the initial NDA submission for the Phase 3 trials and expanded parts of Phase 2 trials SURVEYOR-2 and MAGELLAN-1.

Available SVR12/SVR24 concordance data from Phase 2 trials SURVEYOR-1, SURVEYOR-2 and MAGELLAN-1 are summarized in Table 30 (FDA analysis). Among 547 subjects with SVR12 (observed at Post-Treatment Week 12, i.e., not imputed) who had available data at the Post-Treatment Week 24 visit to assess SVR24, all 547 (100%) also achieved SVR24.

Table 30. Concordance of available SVR12/SVR24 results in Phase 2 trials SURVEYOR-1, SURVEYOR-2 and MAGELLAN-1.

Trial	Total N	N SVR12 (observed)	SVR12 Subjects w/SVR24 data (observed)	N with SVR24=Y (observed)	SVR12/SVR24 Concordance
SURVEYOR-1	174	170	166	166	100%
SURVEYOR-2	691	655	334	334	100%
MAGELLAN-1	141	127	47	47	100%
Pooled Phase 2 Trials	1006	952	547	547	100%

In the 4-month NDA safety update (SDN 26), the sponsor included a brief summary of available follow-up HCV RNA results from 8 ongoing trials based on a database cutoff of 1/4/2017. According to the sponsor, there were no additional relapses observed since the Original NDA submission. Three subjects were determined to be reinfected with HCV, all of whom initially had HCV GT3 infection. One of these 3 subjects (M13594-37739-161301) was reported in the initial NDA submission to have been reinfected with another phylogenetically distinct GT3 strain at the SVR12 assessment; see Section 5.1 for more details. Two other subjects (M14868-46903-1012 and M14868-16140-6013) achieved SVR12 and were later shown to be infected with a different HCV genotype or subtype at the SVR24 assessment. As noted above based on data reported in the initial NDA submission, one subject from ENDURANCE-2 had possible late relapse based on an HCV RNA measurement of 222 IU/mL at Post-Treatment Week 24. In summary, among 2,154 subjects who achieved SVR12 in a Phase 2 or Phase 3 trial and had available data in an SVR24 analysis window, 1 subject (<0.1%) experienced virologic relapse at the SVR24 timepoint, excluding subjects with suspected reinfection.

(b) (4)

5. GLE/PIB DRUG RESISTANCE IN CLINICAL TRIALS (NS3/4A PI-, NS5A Inhibitor-Naïve Subjects)

5.1 Overview of Available Data and Resistance Analysis Methods

Table 31 (FDA analysis) provides a summary of available resistance analysis data from trials that included NS3/4A PI- and NS5A inhibitor-naïve subjects. Across all of these 8 Phase 2b/3 trials, resistance data in any drug target, and for any timepoint, were provided from 2,552/2,646 (96%) subjects. Baseline and post-baseline resistance data were provided for the relevant drug targets in 32/32 (100%) of subjects who experienced virologic failure (on-treatment VF or relapse); 31 subjects had data reported for NS3/4A and NS5A, and 1 subject who received DCV+SOF (no NS3/4A PI) had only NS5A data provided.

Table 31. Summary of available resistance data (NGS analysis of NS3/4A and/or NS5A) submitted for for trials that included NS3/4A PI- and NS5A inhibitor-naïve subjects. Summary includes updated data from M13-594 submitted in SDN 5.

STUDY	SVR12	VF Cat	n	Subjects w/Any Resistance Data	Baseline		Post-Baseline	
					NS3/4A	NS5A	NS3/4A	NS5A
M13-590/ ENDURANCE-1 (GT1, noncirr.)	N	Non-VF	3	100% (3/3)	100% (3/3)	100% (3/3)	33% (1/3)	33% (1/3)
		On-Tx VF	1	1/1	1/1	1/1	1/1	1/1
	Y	n/a	699	97% (676/699)	96% (674/699)	95% (665/699)		
M15-464/ ENDURANCE-2 (GT2, noncirr.)	N	Non-VF	1	1/1	1/1	1/1	0/1	0/1
	Y	n/a	201	94% (189/201)	84% (169/201)	91% (182/201)		
M13-583/ ENDURANCE-4 (GT4-6, noncirr.)	N	Non-VF	1	1/1	1/1	1/1	0/1	0/1
	Y	n/a	120	91% (109/120)	90% (108/120)	90% (108/120)		
M13-594/ ENDURANCE-3 (GT3, noncirr., TN)	N	Non-VF	12	100% (12/12)	75% (9/12)	100% (12/12)	8% (1/12)	8% (1/12)
		On-Tx VF	2	100% (2/2)	100% (2/2)	100% (2/2)	100% (2/2)	100% (2/2)
		Relapse	9	100% (9/9)	89% (8/9)[1]	100% (9/9)	89% (8/9)[1]	100% (9/9)
	Y	n/a	482	98% (474/482)	76% (364/482)[1]	98% (474/482)		
M14-172/ EXPEDITION-1 (cirr, non-GT3)	N	Relapse	1	1/1	1/1	1/1	1/1	1/1
	Y	Missing	145	97% (141/145)	92% (134/145)	96% (139/145)		
M15-462/ EXPEDITION-4 (GT1-6, renal imp.)	N	Non-VF	2	50% (1/2)	50% (1/2)	50% (1/2)	50% (1/2)	50% (1/2)
	Y	n/a	102	93% (95/102)	89% (91/102)	89% (91/102)		
M14-867/ SURVEYOR-1 (Ph2b, GTs1,4-6)	N	Non-VF	1	1/1	1/1	1/1	0/1	0/1
		Relapse	2	100% (2/2)	100% (2/2)	100% (2/2)	100% (2/2)	100% (2/2)
	Y	n/a	171	99% (169/171)	99% (169/171)	99% (169/171)		
M14-868/ SURVEYOR-2 (Ph2b, non-GT1)	N	Non-VF	13	92% (12/13)	92% (12/13)	92% (12/13)	8% (1/13)	15% (2/13)
		On-Tx VF	4	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)
		Relapse	13	100% (13/13)	100% (13/13)	100% (13/13)	100% (13/13)	100% (13/13)
	Y	n/a	661	96% (636/661)	95% (629/661)	96% (633/661)		

[1]NS3/4A sequence analysis data were not provided for subjects in the DCV + SOF control arm as these subjects did not receive and NS3/4A PI.

The resistance analyses considered subjects who received to-be-marketed dose levels of GLE/PIB ± RBV, and censored subjects who did not achieve SVR12 for reasons unrelated to virologic failure (i.e., "non-VF-censored").

One additional subject was censored, M13594-37739-161301, who did not achieve SVR12 due to a hypothesized reinfection based on the sponsor's phylogenetic analyses of HCV NS3/4A and NS5A nucleotide sequences from samples collected at baseline and at the time of presumed virologic failure. This subject had HCV GT3a infection, received GLE/PIB for 8 weeks in ENDURANCE-3, and maintained HCV RNA <LLOQ Target Not Detected through Post-Treatment Week 8. However, at Post-Treatment Week 12 the subject had an HCV RNA level of 7,040 IU/mL, and a confirmatory assessment 6 days later indicated an HCV RNA level of 87,800 IU/mL. To this reviewer's knowledge the sponsor did not comment whether the subject had any behavioral risk factors for HCV reinfection. At the time of virologic failure the HCV subtype (3a) was the same as the subtype at baseline, and also carried an NS5A Y93H substitution. While the presence of the same HCV subtype with an emergent NS5A Y93H substitution is consistent with resistance selection due to DAA treatment, no treatment-emergent NS3 RASs at key resistance-associated positions were detected, and NS5A Y93H has been observed as a natural polymorphism. Furthermore, the sponsor's phylogenetic analyses demonstrated that NS3/4A and NS5A nucleotide sequences from baseline and virologic failure isolates did not cluster as they did for other subjects who experienced virologic failure (Figure 3, Report 0492 pgs. 67-68). An independent analysis of the number of treatment-emergent substitutions in Subject M13594-37739-161301 relative to other virologic failure subjects is consistent with the sponsor's conclusion: the median number of emergent substitutions (detected at >15% frequency) among virologic failure subjects other than M13594-37739-161301 was 1 (range 0-4) for NS3/4A and 1 (range 0-7) for NS5A, while Subject M13594-37739-161301 had 9 and 15 emergent substitutions at >15% frequency in NS3/4A and NS5A, respectively. While one could argue conservatively that the subject could be considered in the treatment-emergent resistance analyses, censoring this subject does not have a significant impact on the overall results.

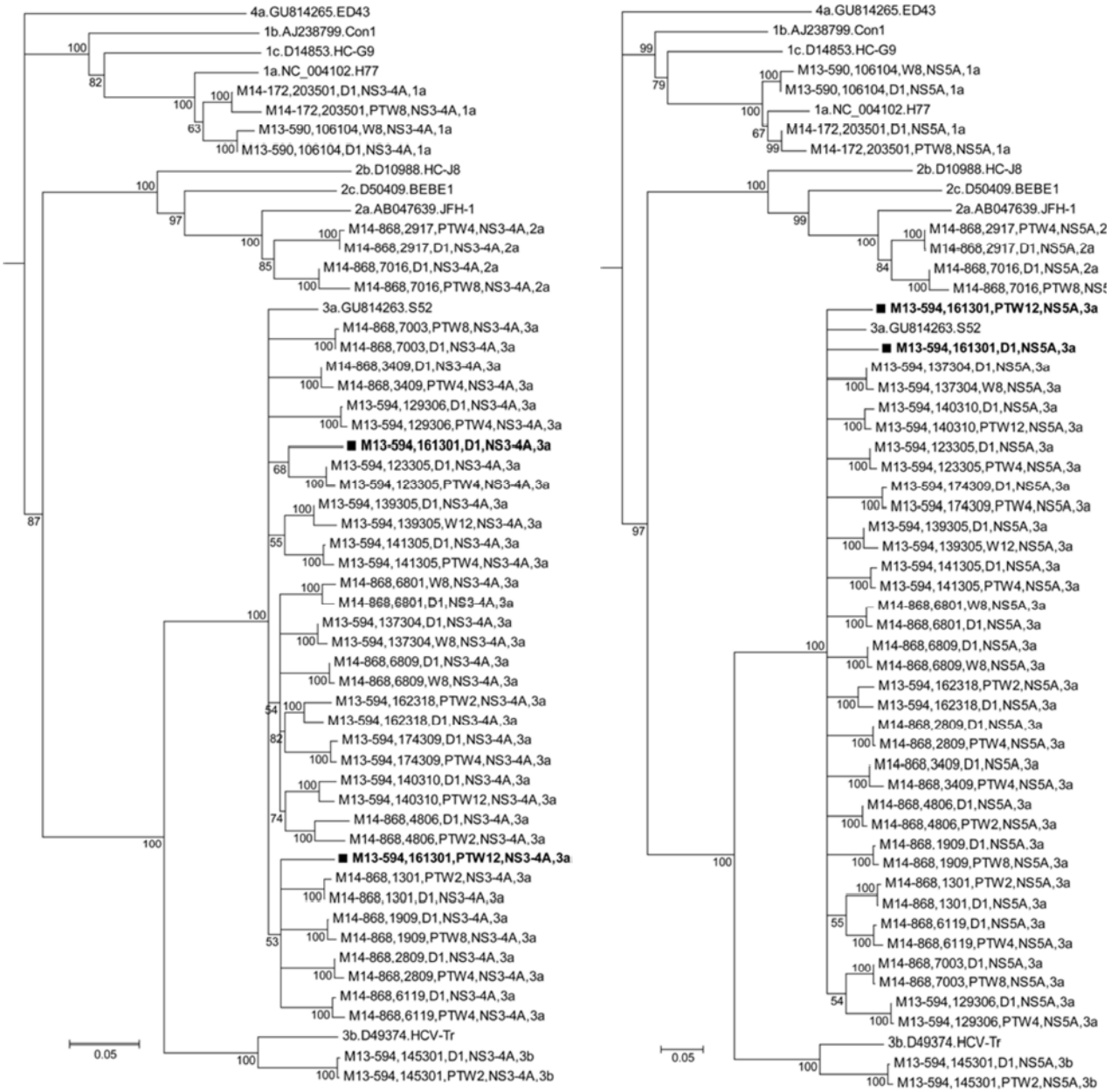


Figure 3. Sponsor's phylogenetic analysis of baseline and post-baseline nucleotide sequences from subjects who experienced virologic failure in Phase 2b/3 trials (excluding MAGELLAN-1). NS3/4A sequences are in the left panel, NS5A sequences are in the right panel. Subject M13594-37739-161301 with hypothesized reinfection is shown in bold.

The resistance analyses described below primarily considered a pre-defined list of known DAA resistance-associated amino acid positions in the NS3 and NS5A drug targets (Table 32, FDA list). These are also the same positions considered for the MAGELLAN-1 trial that included NS3/4A PI- or NS5A inhibitor-experienced subjects (See Section 6). In some cases narrower lists of specific positions/substitutions were considered as guided by the analyses. The same positions were considered for all HCV GTs, except that NS3 positions Q80 and A166 were considered specifically only for HCV GT3. As shown in Section 2.2, substitutions at these positions were shown to reduce the activity of GLE in cell culture for GT3, and a small number of HCV GT3

infected subjects who experienced virologic failure with GLE/PIB had treatment-emergent changes at position Q80.

A separate independent analysis of all reported NS3/4A and NS5A positions was also conducted to assess if substitutions at other positions were associated with virologic failure, but in general this analysis did not identify any clear novel treatment-emergent resistance-associated substitutions, with the possible exception of NS3 I366V in two subjects with HCV GT3a infection (see Section 5.6).

Table 32. NS3 and NS5A DAA resistance-associated positions considered in resistance analyses. NS3 positions in bold font were the primary positions considered in baseline resistance analyses, as these positions are most likely to be associated with resistance to GLE or other NS3/4A PIs. All positions shown were considered in treatment-emergent resistance analyses.

NS3	36, 43, 54, 55, 56, 80 (GT3 only), 107, 122, 132, **155**, **156**, 158, 166 (GT3 only), **168**, 170
NS5A	24, 28, 29, 30, 31, 32, 58, 92, 93

All amino acid sequence data were aligned and reported relative to subtype-specific reference sequences. The subtype-specific references used and reference amino acid sequences at positions of interest are shown in Appendix D.

Baseline resistance analyses primarily considered polymorphisms that were detected at a ≥15% frequency by NGS. Treatment-emergent resistance analyses considered any substitutions that were either emergent or enriched at a post-baseline timepoint relative to baseline, using the following definitions:

- Treatment-emergent: not detected by NGS (i.e., below the 2% NGS sensitivity cutoff) at baseline and then detected (i.e., ≥2%) at a post-baseline timepoint
- Treatment-enriched; detected by NGS both at baseline and post-baseline, but with a ≥20% enrichment in amino acid frequency at post-baseline relative to baseline (e.g., detected at a 20% frequency at baseline and a 99% frequency at failure)

The following review sub-sections summarize resistance analysis findings according to subjects' HCV GT infection. Appendix E includes a subject-level summary of baseline and treatment-emergent RASs for all subjects who experienced virologic failure in Phase 2b/3 trials, excluding MAGELLAN-1.

5.2 HCV GT1 Resistance Analyses

The near 100% efficacy of GLE/PIB for subjects with HCV GT1 infection indicates that natural baseline resistance-associated polymorphisms did not significantly influence treatment efficacy. Across Phase 2b/3 trials of GLE/PIB at the to-be-marketed dose levels, only 2/889 (0.2%) subjects with HCV GT1 infection experienced virologic failure. Considering the regimens proposed for labeling, among noncirrhotic HCV GT1 infected subjects treated with GLE/PIB for 8 weeks, 1/387 (0.3%) subjects experienced virologic failure; the subject experienced on-treatment failure and not relapse and thus a longer treatment duration would not have influenced treatment outcome. Among cirrhotic HCV GT1 infected subjects treated with GLE/PIB for 12 weeks, 1/101 (1%) experienced virologic failure.

Table 33 (FDA analysis) summarizes the baseline and post-baseline NS3 and NS5A RASs detected in the 2 HCV GT1 infected subjects who experienced virologic failure. Both subjects had ≥2 treatment-emergent/enriched NS5A RASs, and Subject 106104 had a treatment-emergent NS3 A156V substitution. The treatment-emergent/enriched NS5A RASs included Q30R (both subjects), L31M and H58D/Y (H58D detected in both subjects). Both subjects also had 2 NS5A resistance-associated polymorphisms detected at baseline, although only one of these (Y93N in Subject 203501) was detected at the ≥15% level.

Table 33. HCV resistance characteristics for HCV GT1 infected subjects who experienced virologic failure in Phase 2b/3 trials of GLE/PIB.

USUBJID	Cirr?	Tx Hist	Regimen	VF Cat	VISIT	Target	Subst.	AA Freq	Tx-Emerg	Tx-Enrch
M13590-50890-106104 (GT1a)	N	IFN- or P/R-exp	GLE/PIB 8W	On-Tx VF	BASELINE	NS5A	Q30R	12.47		
							L31M	13.92		
					WEEK 8	NS3/4A	A156V	99.85	Y	
						NS5A	Q30R	98.45		Y
							L31M	99.58		Y
							H58D	97.37	Y	
							H58Y	2.25	Y	
M14172-48171-203501 (GT1a)	Y	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS5A	K24R	6.23		
							Y93N	46.22		
					PTW 8	NS5A	Q30R	87.79	Y	
							H58D	13.51	Y	
							Y93N	99.73		Y

Table 34 (FDA analysis) summarizes the numbers of HCV GT1 infected subjects with baseline amino acid polymorphisms detected at a ≥15% frequency at DAA resistance-associated positions in NS3 and NS5A, and their treatment outcomes. Table 35 (FDA analysis) summarizes SVR12 rates according to the detection of baseline polymorphisms considering specific amino acid position lists of interest.

Considering the key NS3 resistance-associated positions R155, A156 and D168, no HCV GT1 infected subjects had a polymorphism at position A156, and only 9/845 (1.1%) subjects had a polymorphism at position R155 or D168; all 9 achieved SVR12. Considering 9 resistance-associated positions in NS5A (K/Q24, M/L28, P29, Q/R30, L31, P32, H58, A92, Y93), polymorphisms were detected in 21% (78/380) and 32% (147/461) of GT1a- and GT1b-infected subjects, respectively. No subjects had polymorphisms at position P29 or P32.

Table 34. HCV GT1 infected subjects with baseline HCV resistance-associated polymorphisms (≥15% frequency, non-VF-censored). PM, polymorphism; VF, virologic failure.

						GLE/PIB 12W			GLE/PIB 8W
						Noncirrhotic	Cirrhotic		Noncirrhotic
HCV Subtype	Reference	Target	Position	PM	N	SVR12	VF	SVR12	SVR12
1A n=384 NS3 n=380 NS5A	H77	NS3	155	R155K	4	2	0	0	2
			168	D168E	4	0	0	1	3
		NS5A	24	K24Q	1	0	0	0	1
				K24R	5	3	0	0	2
			28	M28I	1	0	0	1	0
				M28L	1	0	0	1	0
				M28T	2	2	0	0	0
				M28V	29	13	0	2	14
			30	Q30H	6	1	0	2	3
				Q30L	1	0	0	0	1
				Q30R	1	0	0	0	1
			31	L31M	8	2	0	3	3
			58	H58D	1	0	0	0	1
				H58L	1	1	0	0	0
				H58P	14	7	0	1	6
				H58Q	1	0	0	0	1
				H58R	5	1	0	1	3
				H58Y	4	2	0	2	0
			92	A92P	1	0	0	0	1
			93	Y93C	3	2	0	0	1
				Y93H	3	1	0	0	2
				Y93N	3	1	1	0	1
1B N=461 NS3 N=461 NS5A	CON1	NS3	168	D168E	1	0	0	0	1
		NS5A	24	Q24K	2	0	0	0	2
				Q24R	3	2	0	1	0
			28	L28M	11	3	0	0	8
			30	R30K	3	2	0	1	0
				R30L	1	1	0	0	0
				R30M	1	1	0	0	0
				R30Q	17	6	0	3	8
			31	L31I	2	2	0	0	0
				L31M	20	10	0	2	8
			58	P58A	4	3	0	0	1
				P58L	3	3	0	0	0
				P58Q	6	4	0	0	2
				P58R	2	0	0	0	2
				P58S	27	10	0	0	17
				P58T	5	2	0	1	2
			92	A92E	5	1	0	1	3
				A92T	23	14	0	1	8
				A92V	7	4	0	1	2
			93	Y93F	1	0	0	0	1
				Y93H	36	18	0	6	12
				Y93S	1	1	0	0	0
1G N=1 NS3/NS5A	1804	NS5A	24	S24R	1	0	0	1	0
			30	R30Q	1	0	0	1	0
1-Unknown N=1 NS3/NS5A	CON1	NS5A	24	Q24K	1	0	0	0	1
			30	R30T	1	0	0	0	1
			31	L31M	1	0	0	0	1
			93	Y93H	1	0	0	0	1

Table 35. Summary of SVR12 rates for HCV GT1 infected subjects with baseline HCV resistance-associated polymorphisms considering specific amino acid position lists (≥15% frequency, non-VF-censored). ND, no data

HCV Subtype	Resistance-Associated Polymorphism Category	% (n/N)	GLE/PIB 12W (SVR12)		GLE/PIB 8W (SVR12)
			Noncirrhotic	Cirrhotic	Noncirrhotic
GT1a	Any NS3 (R155, A156, D168)	2% (8/384)	100% (2/2)	1/1	100% (5/5)
	Any NS5A RAP (K24,M28,P29,Q30,L31,P32,H58,A92,Y93)	21% (78/380)	100% (30/30)	92% (11/12)	100% (36/36)
	1 NS5A RAP	18% (68/380)	100% (26/26)	90% (9/10)	100% (32/32)
	2+ NS5A RAP	3% (10/380)	100% (4/4)	100% (2/2)	100% (4/4)
GT1b	Any NS3 (R155, A156, D168)	0.2% (1/461)	ND	ND	1/1
	Any NS5A RAP (Q24,L28,P29,R30,L31,P32,P58,A92,Y93)	32% (147/461)	100% (71/71)	100% (11/11)	100% (65/65)
	1 NS5A RAP	26% (120/461)	100% (58/58)	100% (6/6)	100% (56/56)
	2+ NS5A RAP	6% (27/461)	100% (13/13)	100% (5/5)	100% (9/9)

Table 36 (FDA analysis) summarizes SVR12 rates according to the detection of baseline NS5A polymorphisms considering two additional sets of NS5A positions that have been considered in analyses of other NS5A inhibitor-containing DAA programs. In particular, the NS5A "Primary List" includes the positions that should be evaluated for the presence of polymorphisms for HCV GT1a infected patients considering treatment with elbasvir/grazoprevir (Zepatier™ label) or daclatasvir plus sofosbuvir (GT1a patients with cirrhosis; Daklinza™ label). As expected, results considering the "Extended List" are identical to the broader "Any NS5A RAP" list analyses shown above in Table 35, as no GT1 subjects in the dataset had NS5A polymorphisms at positions P29 or 32-the only positions in the "Any NS5A RAP" list that are not in the "Extended List".

Table 36. SVR12 rates for HCV GT1 infected subjects with or without baseline HCV resistance-associated polymorphisms, considering "Primary" and "Extended" NS5A amino acid position lists (≥15% frequency, non-VF-censored).

HCV Subtype	Resistance-Associated Polymorphism Category	GLE/PIB 12W (SVR12)		GLE/PIB 8W (SVR12)
		Noncirrhotic	Cirrhotic	Noncirrhotic
GT1a	With PM: NS5A-Primary List (M28,Q30,L31,Y93)	100% (20/20)	90% (9/10)	100% (22/22)
	No PM: NS5A-Primary List (M28,Q30,L31,Y93)	100% (137/137)	100% (43/43)	99% (147/148)
	With PM: NS5A-Extended List (K24,M28,Q30,L31,H58,A92,Y93)	100% (30/30)	92% (11/12)	100% (36/36)
	No PM: NS5A-Extended List (K24,M28,Q30,L31,H58,A92,Y93)	100% (127/127)	100% (41/41)	99% (133/134)
GT1b	With PM: NS5A-Primary List (L28,R30,L31,Y93)	100% (41/41)	100% (8/8)	100% (34/34)
	No PM: NS5A-Primary List (L28,R30,L31,Y93)	100% (184/184)	100% (36/36)	100% (158/158)
	With PM: NS5A-Extended List (Q24,L28,R30,L31,P58,A92,Y93)	100% (71/71)	100% (11/11)	100% (65/65)
	No PM: NS5A-Extended List (Q24,L28,R30,L31,P58,A92,Y93)	100% (154/154)	100% (33/33)	100% (127/127)

The only specific NS3 or NS5A polymorphism detected at the ≥15% NGS sensitivity level that was associated with virologic failure was NS5A Y93N, which was detected in only 3/380 (<1%) GT1a infected subjects, of whom one experienced virologic failure. Ten other subjects with HCV GT1a infection had ≥2 NS5A resistance-associated polymorphisms detected at the ≥15% level, and all 10 achieved SVR12 (Table 37, FDA analysis).

Taken together, in rare cases NS5A polymorphisms may impact the efficacy of GLE/PIB, but in general this regimen is highly effective in HCV GT1a and GT1b infected subjects with or without NS5A resistance-associated polymorphisms.

Table 37. HCV GT1a infected subjects with ≥2 NS5A resistance-associated polymorphisms (by position, 15% amino acid frequency). All 10 subjects achieved SVR12 with the indicated regimen.

USUBJID	Cirrhosis?	Tx History	Regimen	NS5A PM	AA Freq (%)
M13590-04355-130105	N	IFN- or P/R-experienced	GLE/PIB 8W	Q30H	15.5
				Q30L	44.9
				Y93H	56.9
M13590-45122-260103	N	Naïve	GLE/PIB 12W	Q30H	52.5
				Y93H	52.0
				Y93N	26.8
M13590-45400-310107	N	Naïve	GLE/PIB 12W	M28V	64.7
				H58Y	50.7
M13590-45610-312108	N	Naïve	GLE/PIB 12W	M28V	88.8
				H58R	97.8
M13590-47437-120110	N	IFN- or P/R-experienced	GLE/PIB 12W	M28V	95.1
				H58P	99.2
M13590-50862-313110	N	IFN- or P/R-experienced	GLE/PIB 8W	Q30H	18.6
				L31M	16.3
M14172-43524-302507	Y	Naïve	GLE/PIB 12W	M28V	21.4
				H58Y	17.1
M14172-53404-301501	Y	Naïve	GLE/PIB 12W	M28V	42.5
				H58R	40.1
M14867-12641-2314	N	Naïve	GLE/PIB 8W	M28V	99.4
				Q30R	98.1
M14867-51382-3308	N	Naïve	GLE/PIB 8W	M28V	46.1
				H58R	98.4

The GT1a NS3 Q80K polymorphism also did not impact treatment outcome. This polymorphism was detected in 135/384 (35%) subjects, all of whom achieved SVR12 with GLE/PIB for 8 or 12 weeks (data not shown).

5.3 HCV GT2 Resistance Analyses

As with HCV GT1 infected subjects, the near 100% efficacy of GLE/PIB for subjects with HCV GT2 infection indicates that natural baseline resistance-associated polymorphisms did not significantly affect treatment outcome. Across Phase 2b/3 trials of GLE/PIB at to-be-marketed dose levels, only 2/466 (0.4%) GT2 subjects experienced virologic failure. Considering the regimens proposed for labeling, among noncirrhotic HCV GT2 infected subjects treated with GLE/PIB for 8 weeks, 2/197 (1%) subjects experienced virologic failure; both failures were due to relapse and occurred in treatment-experienced subjects. As noted in Section 4.8, one of these two relapsers had low GLE exposure, possibly related to a medical history of gastric bypass. All 35 cirrhotic HCV GT2 infected subjects treated with GLE/PIB for 12 weeks achieved SVR12.

As noted above in the individual trial analyses, several HCV GT2 subtypes were represented in Phase 2b/3 trials, with subtypes 2a, 2b and 2c being most common. Table 38 (FDA analysis) provides a pooled efficacy summary according to HCV GT2 subtype (derived).

Table 38. Pooled efficacy results for HCV GT2 infected subjects according to GT2 subtype (derived subtype result, prioritizing NS3/NS5A phylogenetic analysis results; non-VF-censored).

HCV GT2 Subtype	Cirrhosis?	Regimen	SVR12
2a	N	GLE/PIB 8W	94% (29/31)
		GLE/PIB 12W	100% (76/76)
	Y	GLE/PIB 12W	100% (4/4)
2a/2c	N	GLE/PIB 12W	100% (3/3)
2a/c	N	GLE/PIB 8W	100% (5/5)
2b	N	GLE/PIB 8W	100% (146/146)
		GLE/PIB 12W	100% (77/77)
	Y	GLE/PIB 12W	100% (27/27)
2c	N	GLE/PIB 8W	100% (6/6)
		GLE/PIB 12W	100% (47/47)
	Y	GLE/PIB 12W	100% (2/2)
2i	N	GLE/PIB 12W	100% (7/7)
2k	N	GLE/PIB 12W	1/1
2l	N	GLE/PIB 12W	100% (3/3)
2q	N	GLE/PIB 8W	1/1
		GLE/PIB 12W	100% (2/2)
2t	N	GLE/PIB 12W	1/1
2-unknown	N	GLE/PIB 8W	100% (6/6)
		GLE/PIB 12W	100% (15/15)
	Y	GLE/PIB 12W	100% (2/2)

Table 39 (FDA analysis) summarizes the baseline and post-baseline NS3 and NS5A RASs detected in the 2 HCV GT2 infected subjects who experienced virologic failure. Both subjects had a subtype 2a infection, with NS3 I132L and NS5A L31M detected at baseline and at the time of relapse, and no treatment-emergent or treatment-enriched substitutions at known resistance-associated positions. While both subjects had an NS3 I132L polymorphism relative to the prototypic subtype 2a reference strain JFH-1, it is arguably not a real polymorphism as L132 was the consensus sequence at this position, with 103/104 (99%) GT2a subjects having an I132L change relative to the JFH-1 reference. Similarly, NS5A M31 is highly common in subtype 2a and the sequence at this position just happens to differ between the prototypic reference and the consensus for this subtype; 102/109 (94%) HCV GT2a infected subjects with available data had the M31 sequence at baseline. Interestingly, the converse technical issue occurred in subtype 2b, where the reference was M31 yet most subjects with subtype 2b had L31 detected.

Table 39. HCV resistance characteristics for HCV GT2 infected subjects who experienced virologic failure in Phase 2b/3 trials of GLE/PIB.

GT2 Subtype	USUBJID	BL HCV RNA (IU/mL)	Cirr?	Tx Hist	Regimen	VF Cat	VISIT	Target	Subst.	AA Freq
2A	M14868-22887-2917	2,870,000	N	SOF-exp (+/- IFN or RBV)	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	I132L	99.55
								NS5A	L31M	99.44
							PTW 4	NS3/4A	I132L	99.84
								NS5A	L31M	99.93
	M14868-32999-7016	13,200,000	N	IFN- or P/R-exp	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	I132L	99.34
								NS5A	L31M	99.69
							PTW 8	NS3/4A	I132L	99.67
								NS5A	L31M	99.87

Table 40 (FDA analysis) summarizes the frequency of baseline amino acid polymorphisms detected at a ≥15% frequency at DAA resistance-associated positions in NS3 and NS5A (positions indicated in Table 32), and

treatment outcomes. This summary censored 5 subjects with unknown HCV GT2 subtypes due to inconsistent reference use. Overall, baseline polymorphisms at the key NS3 resistance-associated positions R155, A156 and D168 were detected only in 3/400 (0.8%) subjects. Baseline NS5A resistance-associated polymorphisms were detected in 329/413 (80%) subjects. As noted above, the high prevalence of NS5A polymorphisms is largely attributed to differences at position 31 between the subtype-specific consensus sequences and the prototypic reference strains used.

Table 40. HCV GT2 infected subjects with baseline HCV resistance-associated polymorphisms (according to subtype reference, ≥15% frequency, non-VF-censored). Subjects with multiple polymorphisms are counted multiple times for each individual polymorphism shown. ND, no data; PM, polymorphism; VF, virologic failure. Data for 5 subjects with unknown GT2 subtypes and inconsistent reference use are not included in this table.

						GLE/PIB 12W		GLE/PIB 8W	
						Noncirrhotic	Cirrhotic	Noncirrhotic	
HCV Subtype	**Reference**	**Target**	**Position**	**PM**	**N**	**SVR12**	**SVR12**	**VF**	**SVR12**
2a N=104 NS3 N=109 NS5A	JFH-1	NS3	168	D168E	2	0	1	0	1
		NS5A	24	T24A	9	2	2	0	5
				T24S	1	0	0	0	1
			28	F28C	1	1	0	0	0
				F28L	2	2	0	0	0
				F28V	1	1	0	0	0
			30	K30R	2	1	0	0	1
			31	L31M	102	68	4	2	28
				L31V	1	1	0	0	0
			58	P58S	3	2	0	0	1
			92	C92S	3	3	0	0	0
2b N=239 NS3 N=236 NS5A	HC-J8	NS3	168	D168V	1	1	0	0	0
		NS5A	28	L28F	8	5	1	0	2
			30	K30R	1	1	0	0	0
			31	M31I	2	2	0	0	0
				M31L	159	44	19	0	96
				M31V	1	0	0	0	1
			58	P58A	1	0	0	0	1
				P58S	10	2	1	0	7
			92	C92S	3	1	1	0	1
2c N=48 NS3 N=55 NS5A	BEBE1	NS5A	24	S24A	1	1	0	0	0
			28	F28C	21	20	0	0	1
			30	R30K	49	42	2	0	5
			31	L31F	2	2	0	0	0
				L31M	10	9	0	0	1
			58	P58A	1	1	0	0	0
			92	C92W	1	0	1	0	0
2i N=6 NS3 N=7 NS5A	D54	NS5A	28	F28L	1	1	0	0	0
			30	K30R	2	2	0	0	0
2l N=2 NS3 N=3 NS5A	MRS89	NS5A	92	S92C	1	1	0	0	0
2q N=1 NS3/NS5A	852	NS5A	24	S24T	1	0	0	0	1
			28	L28F	1	0	0	0	1
2t N=1 NS5A	MRS40	NS5A	28	L28F	1	1	0	0	0
			58	P58T	1	1	0	0	0

Taken together, baseline HCV polymorphisms did not influence GLE/PIB treatment outcome in subjects with HCV GT2 infection. The two HCV GT2 infected subjects who experienced virologic failure did not have virus at

baseline with any unusual amino acid sequences at known resistance-associated positions. Furthermore, no treatment-emergent substitutions at known resistance-associated positions were detected at the time of failure.

5.4 HCV GT3 Resistance Analyses

Pooled efficacy results for HCV GT3 infected subjects across Phase 2b/3 trials are summarized in Figure 4 (FDA analysis). SVR12 rates (non-VF-censored) ranged from 90-100%, with some evidence that longer treatment durations were slightly more effective, although the differences across various treatment regimens within the same study population were small. Due to limited data and a lack of an obvious impact of RBV on treatment outcome, subjects treated with the GLE/PIB + RBV regimen were excluded from subsequent analyses.

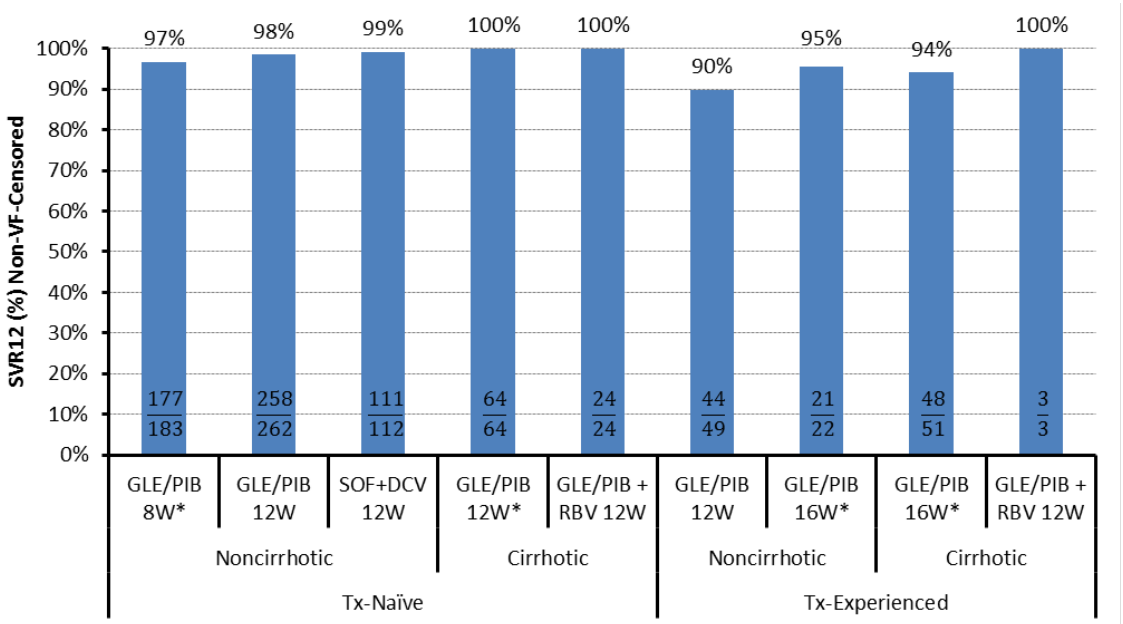


Figure 4. Summary of SVR12 rates (non-VF-censored) for HCV GT3 infected subjects. *Sponsor's proposed recommended regimen in label. Note that the 98% (258/262) SVR12 rate for GLE/PIB 12W in treatment-naïve, noncirrhotic subjects includes one subject (M13594-37739-161301) who had suspected reinfection to explain an observation of virologic relapse (see Section 5.1 for details); this subject was excluded from the subsequent analyses below.

The modest differences in SVR12 rates according to GLE/PIB treatment duration are largely attributed to differences in relapse rates (Table 41; FDA analysis).

Table 41. Pooled summary of virologic outcomes (non-VF-censored) among HCV GT3 infected subjects. Subject M13594-37739-161301 with suspected reinfection was censored in this analysis. *Label-proposed regimens.

Tx History	Cirrhosis?	Regimen	N	SVR12	On-Tx VF	Relapse	Relapse Rate (non-VF- and on-Tx-VF-censored)
Tx-Naïve	N	GLE/PIB 8W*	183	177	1	5	2.7% (5/182)
		GLE/PIB 12W	261	258	1	2	0.8% (2/260)
		SOF+DCV 12W	112	111	0	1	0.9% (1/112)
	Y	GLE/PIB 12W	64	64	0	0	0% (0/64)
Tx-Experienced	N	GLE/PIB 12W	49	44	1	4	8.3% (4/48)
		GLE/PIB 16W*	22	21	0	1	4.5% (1/22)
	Y	GLE/PIB 16W	51	48	1	2	4.0% (2/50)

Across all GLE/PIB treatment regimens (to-be-marketed doses) in the non-VF-censored dataset, 620/630 (98%) HCV GT3 infected subjects had a subtype 3a infection, and the remaining subjects had subtype 3b (n=7), 3g (n=1), or 3i (n=2). Overall, 90% (9/10) non-GT3a subjects achieved SVR12; one GT3b infected subject (M13594-47009-145301, treatment-naïve, noncirrhotic) experienced virologic relapse after treatment with GLE/PIB for 12 weeks. Due to the limited number of subjects with non-3a subtype infection it is not possible to draw any major conclusions about treatment efficacy for these subtypes.

As in other HCV GTs, across different GT3 subtypes there is some amino acid variability at certain resistance-associated positions in NS3 and NS5A (Table 42 and Table 43, respectively; FDA analyses). Key NS3/4A PI resistance-associated positions in NS3, including R155, A156 and Q168, were highly conserved, with 15/742 (2%) subjects having virus with Q168K or Q168R polymorphisms (including 1 subject with both detected), and no subjects with polymorphisms at the other two positions. Among non-VF-censored subjects who received GLE/PIB for 8, 12 or 16 weeks, baseline Q168K/R polymorphisms were detected in 1.6% (10/620) of subjects. Of note, the wild-type amino acid at position 168 differs between GT3 (Q168) and GT1 (D168), which explains why some NS3/4A PIs have poor activity against HCV GT3 (e.g., simeprevir; Lenz et al., 2013).

For NS5A, as noted below in the resistance analyses baseline or treatment-emergent NS5A A30K and Y93H were associated with GLE/PIB treatment failure. Among HCV GT3a infected subjects, A30-any, A30K and Y93H were detected in 93/857 (11%), 53/857 (6%) and 48/857 (6%) subjects, respectively; 100/857 (12%) subjects had A30K or Y93H detected, and 1 subject had both detected (M13594-45400-139305, GLE/PIB 12W on-treatment failure). For subtypes 3b, 3g and 3i a lysine (K) is the wild-type reference sequence at position 30. As noted above, due to a limited number of subjects one cannot draw any major conclusions about GLE/PIB treatment efficacy for non-3a subtypes; however, the presence of NS5A K30 raises the theoretical possibility that efficacy could be suboptimal in these other subtypes. Considering all NS5A resistance-associated positions (Table 32), 22% (137/622; non-VF-censored) of GT3-infected subjects who received GLE/PIB for 8, 12 or 16 weeks had virus with baseline NS5A resistance-associated polymorphisms.

Table 42. NS3 polymorphisms observed according to GT3 subtype in Phase 2b/3 trials (15% NGS cutoff, or Sanger population sequencing; all subjects in resistance dataset with baseline data).
Subtype-specific reference strains were used for resistance analyses.

GT3 Subtype	N w/ data	Ref Strain	NS3 Position and Reference AAs[1]											
			43	54	55	56	80	107	122	132	158	166	168	170
3a	742	S52	F	T	V	Y	Q	V	S	L	V	A	Q	V
			L (n=1)	A (n=1), S (n=4)	I (n=2)	F (n=1)	K (n=1)	I (n=24)	A (n=1), C (n=2), T (n=6)	I (n=113), V (n=1)	I (n=1)	S (n=66), T (n=37)	K (n=10), R (n=6)	I (n=711)
3b	6	HCV-Tr	F	T	V	Y	Q	V	S	L	V	A	Q	I
3g	0	BID-G1243	F	T	V	Y	Q	V	S	L	V	S	Q	I
3i	3	IND-HCV-3i	F	T	V	Y	Q	V	S	I	V	A	Q	V
										L (n=1)	I (n=1)			

[1]Positions L36, R155 and A156 are not shown as they were 100% conserved across reference strains and clinical isolates.

Table 43. NS5A polymorphisms observed according to GT3 subtype in Phase 2b/3 trials (15% NGS cutoff, or Sanger population sequencing; all subjects in resistance dataset with baseline data).
Subtype-specific reference strains were used for resistance analyses.

GT3 Subtype	N w/ data	Ref Strain	NS5A Position and Reference AAs								
			24	28	29	30	31	32	58	92	93
3a	857	S52	S	M	P	A	L	P	P	E	Y
			A (n=15)	V (n=8)		E (n=1), K (n=53), L (n=1), M (n=3), R (n=1), S (n=20), T (n=10), V (n=11)			A (n=8), R (n=6), S (n=11), T (n=3)	D (n=1), G (n=1)	H (n=48)
3b	6	HCV-Tr	S	M	P	K	V	P	P	E	Y
						M (n=1)	M (n=6)				
3g	0	BID-G1243	S	M	P	K	V	P	P	E	Y
			(no data)								
3i	3	IND-HCV-3i	S	M	P	K	L	P	P	E	Y
							M (n=1)				

A detailed subject-level listing of baseline and treatment-emergent polymorphisms/substitutions detected in HCV GT3 infected subjects who experienced virologic failure in Phase 2b/3 trials is provided in Appendix E.

Table 44 (FDA analysis) summarizes SVR12 rates for subjects who had virus with polymorphisms at NS3 and NS5A resistance-associated positions. Results are presented for the various treatment regimens evaluated, but irrespective of prior treatment history or cirrhosis status. Polymorphisms shown in these analyses to be potentially associated with reduced GLE/PIB efficacy were investigated in more detail as discussed below.

Table 44. SVR12 rates for subjects with baseline polymorphisms at NS3 or NS5A resistance-associated positions (15% NGS cutoff, non-VF-censored). Note that in this analysis, a subject is counted multiple times for rare cases when multiple distinct polymorphisms are detected at the same amino acid position at the ≥15% level. ND, no data; n/a, not applicable as DCV+SOF does not include an NS3/4a PI.

Target	Position	Polymorphism	SVR12 Rate			
			GLE/PIB 8W N=181	**GLE/PIB 12W** N=367 (NS3), 369 (NS5A)	**GLE/PIB 16W** N=72	**SOF+DCV 12W** N=110
NS3	43	F43L	ND	1/1	ND	n/a
	54	T54A	1/1	ND	ND	
		T54S	0/1	1/1	ND	
	55	V55I	ND	1/1	ND	
	56	Y56F	ND	1/1	ND	
	80	Q80K	ND	1/1	ND	
	107	V107I	100% (6/6)	92% (11/12)	100% (2/2)	
	122	S122C	1/1	ND	ND	
		S122T	100% (4/4)	1/1	ND	
	132	I132L (GT3i)	ND	1/1	ND	
		L132I	100% (22/22)	96% (46/48)	100% (17/17)	
		L132V	ND	1/1	ND	
	158	V158I	1/1	ND	ND	
	166	A166S	82% (14/17)	97% (31/32)	75% (6/8)	
		A166T	100% (8/8)	100% (19/19)	100% (3/3)	
	168	Q168K	1/1	100% (5/5)	ND	
		Q168R	0/1	50% (1/2)	1/1	
	170	V170I	96% (158/164)	98% (343/350)	94% (64/68)	
NS5A	24	S24A	100% (4/4)	100% (8/8)	ND	100% (3/3)
	28	M28V	100% (2/2)	100% (4/4)	1/1	ND
	30	A30K	78% (14/18)	79% (15/19)	0/1	100% (5/5)
		A30L	ND	1/1	ND	ND
		A30M	1/1	100% (2/2)	ND	ND
		A30R	ND	1/1	ND	ND
		A30S	100% (7/7)	100% (6/6)	100% (2/2)	100% (4/4)
		A30T	100% (2/2)	100% (6/6)	1/1	ND
		A30V	100% (3/3)	80% (4/5)	100% (2/2)	ND
		K30M (GT3b)	ND	ND	1/1	ND
	31	V31M (GT3b)	100% (2/2)	50% (1/2)	100% (2/2)	ND
	58	P58A	100% (4/4)	100% (3/3)	ND	1/1
		P58R	ND	100% (4/4)	1/1	ND
		P58S	100% (5/5)	100% (3/3)	ND	ND
		P58T	100% (2/2)	ND	ND	ND
	92	E92D	ND	1/1	ND	ND
		E92G	ND	1/1	ND	ND
	93	Y93H	100% (10/10)	85% (17/20)	1/1	88% (7/8)

Table 45 and Table 46 (FDA analyses) summarize additional analyses into the potential impact of HCV GT3 resistance-associated polymorphisms on treatment efficacy for treatment-naïve and treatment-experienced subjects, respectively.

Table 45. SVR12 rates for treatment-naïve subjects with certain baseline resistance-associated polymorphisms (15% NGS cutoff, non-VF-censored). *sponsor-proposed recommended GLE/PIB regimen; ^Subject M13594-45400-139305 had NS5A A30K and Y93H polymorphisms detected and experienced on-treatment VF. ND, no data; PM, polymorphism. Highlighted rows indicate key polymorphisms of interest.

	Tx-Naïve, No Cirrhosis				Tx-Naïve, w/Cirrhosis	
	GLE/PIB 8W*		GLE/PIB 12W		GLE/PIB 12W*	
Polymorphism	With PM	Without PM	With PM	Without PM	With PM	Without PM
NS3 T54A/S	50% (1/2)	97% (174/179)	1/1	99% (251/254)	ND	100% (63/63)
NS3 A166S/T	88% (22/25)	98% (153/156)	100% (33/33)	99% (219/222)	100% (9/9)	100% (54/54)
NS3 A166S	82% (14/17)	98% (161/164)	100% (21/21)	99% (231/234)	100% (6/6)	100% (57/57)
NS3 Q168K/R	50% (1/2)	97% (174/179)	80% (4/5)	99% (248/250)	100% (2/2)	100% (61/61)
NS3 Q168R	0/1	97% (175/180)	50% (1/2)	99% (251/253)	ND	100% (63/63)
NS3-any (54,166,168)	85% (23/27)	99% (152/154)	97% (37/38)	99% (215/217)	100% (10/10)	100% (53/53)
NS5A A/K30-any	87% (26/30)	99% (149/151)	96% (25/26)	99% (229/231)	100% (5/5)	100% (58/58)
NS5A A30K (3a)	78% (14/18)	99% (159/161)	93% (13/14)^	>99% (239/240)	1/1	100% (62/62)
NS5A Y93H	100% (10/10)	96% (165/171)	91% (10/11)^	99% (244/246)	100% (5/5)	100% (58/58)
NS5A Any (full list)	93% (51/55)	98% (124/126)	96% (47/49)	>99% (207/208)	100% (12/12)	100% (51/51)
NS5A Any (30,93)	89% (33/37)	99% (142/144)	97% (33/34)	99% (221/223)	100% (9/9)	100% (54/54)
NS5A Any (24,28,30,31,58,92,93)	93% (51/55)	98% (124/126)	96% (47/49)	>99% (207/208)	100% (12/12)	100% (51/51)
NS5A Any (28,30,31,93)	90% (37/41)	99% (138/140)	95% (35/37)	>99% (219/220)	100% (10/10)	100% (53/53)

Considering the totality of resistance data, there was not a clear association between the detection of NS3 resistance-associated polymorphisms and treatment outcome with GLE/PIB for subjects with GT3 infection. The NS3 polymorphisms T54S and Q168R were observed in a small number of subjects who experienced virologic failure, but it is difficult to interpret the impact of these polymorphisms due to their low prevalence (6 subjects who received GLE/PIB). Furthermore, the contribution of Q168R in 2 virologic failure subjects is confounded by the presence of NS5A resistance-associated polymorphisms (A30K or A30K+Y93H) in both subjects, and in one of these subjects Q168R was no longer detected at the time of failure, indicating that it was not preferentially selected by GLE/PIB treatment (Appendix E). Similarly, NS3 A166S was detected in 6 subjects who experienced virologic failure, but at the time of virologic failure A166S was no longer detected in 2 subjects, and it declined to a 4% level in a third subject, indicating that viruses harboring this polymorphism were not preferentially selected by GLE/PIB treatment. Furthermore, 3 of the 6 subjects with baseline NS3 A166S who experienced virologic failure also had the NS5A A30K polymorphism detected.

In contrast, the totality of baseline and treatment-emergent resistance data indicate that certain NS5A resistance-associated polymorphisms, particularly A30K, can influence GLE/PIB efficacy. Among treatment-naïve, noncirrhotic subjects treated with GLE/PIB for 8 or 12 weeks, 9 subjects experienced virologic failure (excluding 1 suspected reinfection), of whom 5 (56%) had subtype 3a virus with an NS5A A30K polymorphism, including one subject with A30K+Y93H (Table 45). Another virologic failure subject had subtype 3b virus with a "wild-type" NS5A K30 sequence (noncirrhotic, GLE/PIB 12W). Note that of the 4 virologic failure subjects with baseline NS5A A30K in the 8-week treatment group, 3 failures were due to relapse and 1 was due to on-treatment virologic failure; the on-treatment failure subject would not have benefited from a longer treatment duration. Relapse rates for GT3a subjects with the A30K polymorphism were 17.6% (3/17) and 0% (0/13) for subjects who received the 8-week and 12-week durations, respectively. For GT3a subjects without the A30K polymorphism, relapse rates were 1.2% (2/161) and 0.4% (1/240) for subjects who received the 8-week and 12-week durations, respectively.

Similarly, among 9 treatment-experienced subjects who experienced virologic failure with GLE/PIB for 12 or 16 weeks, 6 (67%) subjects had an A30K or Y93H polymorphism (4 w/A30K, 2 w/Y93H) (Table 46). However, insufficient data are available to assess the impact of these polymorphisms specifically on the efficacy of the 16-week duration recommended in the label (n=1 each for A30K or Y93H).

Table 46. SVR12 rates for treatment-experienced subjects with certain baseline resistance-associated polymorphisms (15% NGS cutoff, non-VF-censored). *sponsor-proposed recommended GLE/PIB regimen. ND, no data; PM, polymorphism. Highlighted rows indicate key polymorphisms of interest.

	Tx-Exp., No Cirrhosis				Tx-Exp., w/Cirrhosis	
	GLE/PIB 12W		GLE/PIB 16W*		GLE/PIB 16W*	
Polymorphism	With PM	Without PM	With PM	Without PM	With PM	Without PM
NS3 T54A/S	ND	90% (44/49)	ND	95% (20/21)	ND	94% (48/51)
NS3 A166S/T	88% (7/8)	90% (37/41)	100% (2/2)	95% (18/19)	78% (7/9)	98% (41/42)
NS3 A166S	80% (4/5)	91% (40/44)	100% (2/2)	95% (18/19)	67% (4/6)	98% (44/45)
NS3 Q168K/R	ND	90% (44/49)	ND	95% (20/21)	1/1	94% (47/50)
NS3 Q168R	ND	90% (44/49)	ND	95% (20/21)	1/1	94% (47/50)
NS3 Any (54,166,168)	88% (7/8)	90% (37/41)	100% (2/2)	95% (18/19)	80% (8/10)	98% (40/41)
NS5A A/K30-Any	40% (2/5)	95% (42/44)	50% (1/2)	100% (19/19)	100% (5/5)	93% (43/46)
NS5A A30K (3a)	25% (1/4)	95% (42/44)	0/1	100% (19/19)	ND	94% (47/50)
NS5A Y93H	50% (2/4)	93% (42/45)	ND	95% (20/21)	1/1	94% (47/50)
NS5A Any (full list)	55% (6/11)	100% (38/38)	67% (2/3)	100% (18/18)	100% (7/7)	93% (41/44)
NS5A Any (30,93)	38% (3/8)	100% (41/41)	50% (1/2)	100% (19/19)	100% (5/5)	93% (43/46)
NS5A Any (24,28,30,31,58,92,93)	55% (6/11)	100% (38/38)	67% (2/3)	100% (18/18)	100% (7/7)	93% (41/44)
NS5A Any (28,30,31,93)	50% (5/10)	100% (39/39)	67% (2/3)	100% (18/18)	100% (6/6)	93% (42/45)

Table 47 (FDA analysis) summarizes the SVR12 rates for subjects with NS5A A30K or Y93H polymorphisms, considering only the sponsor's proposed label-recommended regimens.

Table 47. SVR12 rates for subjects with NS5A A30K (GT3a only) or Y93H polymorphisms, considering sponsor's label-recommended regimens (15% NGS cutoff, non-VF-censored). ND, no data.

	Tx-Naïve, No Cirrhosis GLE/PIB 8W	Tx-Naïve, w/Cirrhosis GLE/PIB 12W	Tx-Exp, No Cirrhosis GLE/PIB 16W	Tx-Exp, w/Cirrhosis GLE/PIB 16W	Pooled Sponsor's Proposed Recom. Regimens
BL NS5A A30K (GT3a) (15%)					
Without Polymorphism	99% (159/161)	100% (62/62)	100% (19/19)	94% (47/50)	98% (287/292)
With Polymorphism	78% (14/18)	1/1	0/1	ND	75% (15/20)
BL NS5A Y93H (15%)					
Without Polymorphism	96% (165/171)	100% (58/58)	95% (20/21)	94% (47/50)	97% (290/300)
With Polymorphism	100% (10/10)	100% (5/5)	ND	1/1	100% (16/16)
BL NS5A A30K or Y93H (15%)					
Without Polymorphism	99% (151/153)	100% (57/57)	100% (20/20)	94% (47/50)	98% (275/280)
With Polymorphism	86% (24/28)	100% (6/6)	0/1	1/1	86% (31/36)

Table 48 (FDA analysis) summarizes NS3 and NS5A substitutions that emerged or became enriched in HCV GT3 infected subjects who experienced virologic failure with a GLE/PIB regimen (8, 12 or 16 weeks duration). Overall, most GT3 infected subjects who experienced virologic failure with GLE/PIB had treatment-emergent

RASs in NS3 and NS5A. A variety of different NS3 substitutions emerged, including Y56H/N, Q80K/R, A156G, and Q186L/R. In NS5A, Y93H was the predominant treatment-emergent resistance pathway, occurring in 15/18 (83%) subjects, with one additional subject having predominant (>99%) Y93H at baseline and failure.

Table 48. Treatment-emergent resistance-associated substitutions in HCV GT3 infected subjects who experienced virologic failure with GLE/PIB regimens. Analysis includes HCV amino acid substitutions that were not detected at baseline and detected at any amino acid frequency ≥2% after virologic failure, or substitutions that were detected at baseline but enriched by ≥20% frequency after virologic failure.

Target	Amino Acid Substitution	% (n/N) of subjects with Tx-emergence
NS3	Y56H	33% (6/18)
	Y56N	11% (2/18)
	Y56H/N	**33% (6/18)**
	Q80K	17% (3/18)
	Q80R	11% (2/18)
	Q80K/R	**22% (4/18)**
	V107I	**6% (1/18)[1]**
	A156G	**11% (2/18)**
	A166Y	**6% (1/18)[1]**
	Q168L	17% (3/18)
	Q168R	17% (3/18)
	Q168L/R	**33% (6/18)**
NS5A	**M28G**	**6% (1/18)**
	P29Q	**6% (1/18)[2]**
	A30G	6% (1/18)
	A30K	11% (2/18)
	A30G/K	**17% (3/18)**
	L31F	**11% (2/18)**
	P58Q	6% (1/18)[2]
	P58T	11% (2/18)[2]
	P58Q/T	**11% (2/18)[2]**
	Y93H	**83% (15/18)**
Any NS3/4A RAS		72% (13/18)[1]
Any NS5A RAS		89% (16/18)
Any NS3/4A or NS5A RAS		89% (16/18)
Any NS3/4A AND NS5A RAS		72% (13/18)[1]

[1]NS3 substitutions V107I and A166Y were not convincing treatment-emergent RASs. Excluding these substitutions, 11/18 (61%) subjects had treatment-emergent/-enriched NS3/4A RASs, and all 11 subjects had NS5A RASs. See text for details.
[2]NS5A substitutions P29Q and P58Q/T were not convincing treatment-emergent RASs. See text for details.

Although included in Table 48, the emergence of substitutions NS3 V107I or A166Y were not convincingly associated with treatment failure. NS3 V107I was detected as a treatment-enriched substitution only in one subject (M14868-42362-1909), and this subject had V107I already detected at a relatively high level (41.5%) at baseline; this subject also had predominant (99.6%) NS5A A30K at baseline, as well as treatment-emergent NS5A Y93H. The specific A166Y substitution also emerged only in a single subject (M13594-53762-140310). This subject had an NS3 A166S polymorphism detected at a 99.2% level at baseline, and at a 97.7% level at the time of virologic failure, with A166Y being detected at the same position at a minimal level (2.3%) at the

time of failure to meet the definition of treatment-emergence; NS5A Y93H also emerged in this subject. Furthermore, as noted above, viruses harboring the A166S polymorphism did not appear to be preferentially selected by GLE/PIB treatment in subjects who experienced virologic failure. Excluding these two NS3 substitutions (V107I and A166Y), the total number of subjects with any treatment-emergent NS3 RAS was 11/18 (61%), all of whom also had treatment-emergent NS5A RAS(s).

Similarly, emergence of the NS5A substitutions P29Q and P58Q/T was not convincingly associated with treatment failure. The P29Q substitution emerged only in 1 subject (M14868-43362-6801) at the time of treatment failure at a 2.6% level, just above the minimum detection threshold, while the subject had predominant (>99.5%) A30K plus Y93H detected at the same time. This subject also had NS5A P58Q and P58T substitutions detected at a comparable level (~2%) at the time of failure. A second subject (M14868-12641-1301) had an NS5A P58T substitution detected at a 2.2% level at the time of failure, again with predominant (>99.5%) A30K and Y93H detected at the same time. Excluding NS5A P29Q and P58Q/T does not impact the overall rate of detection of treatment-emergent NS5A RASs.

Consistent with phenotypic analysis results, most virologic failure subjects (14/18, 78%) had multiple NS5A RASs detected at the time of failure, including baseline polymorphisms and treatment-emergent substitutions, with A30G/K + Y93H being most common (11/18, 61%) (Table 49; FDA analysis). In cell culture, A30K and Y93H as single substitutions did not reduce PIB anti-HCV activity (1.1- and 2.3-fold, respectively); however, A30K + Y93H reduced PIB activity by 69-fold. Interestingly, the fact that baseline A30K was more strongly associated with virologic failure compared to Y93H may reflect the easier ability of the virus to acquire an additional Y93H substitution (1 transition nucleotide change) compared to virus with a baseline Y93H acquiring an A30K substitution (2 nucleotide changes, including 1 transversion). One subject who had only a single NS5A RAS at failure had an M28G substitution; no phenotypic analysis data for GT3a are available for this substitution, but in GT1a M28G alone reduced PIB activity by 244-fold.

Importantly, there is no obvious indication that treatment duration (8-16 weeks) influenced the likelihood of detecting NS3 and NS5A RASs at the time of virologic failure (Table 49). Therefore, one should expect a high likelihood of treatment-emergent RASs in GT3-infected patients who fail treatment with any GLE/PIB treatment duration ≥8 weeks.

Table 49. NS3 and NS5A RASs detected at the time of virologic failure in HCV GT3 infected subjects.

Regimen	USUBJID	Subtype	NS3 RAS (bold=tx-emergent, ^{Enr}Tx-enriched)	NS5A RAS (bold=tx-emergent, ^{Enr}Tx-enriched)
GLE/PIB 8W	M13594-07584-174309	3a	**Q168L**	A30K, **Y93H**
	M13594-09818-162318	3a	**Y56H**	A30K, **Y93H**
	M13594-15494-123305	3a	**Y56H**, A166S, **Q168L**	A30K, **Y93H**
	M13594-32999-129306	3a	T54S	(none)
	M13594-45207-137304	3a	**Q80R**, **A156G**	A30K, **Y93H**
	M13594-53762-140310	3a	A166S/**Y**	**Y93H**
GLE/PIB 12W	M13594-45400-139305	3a	**Y56H**, **Q168R**^{Enr}	**A30K**^{Enr}, **Y93H**^{Enr}
	M13594-47009-145301	3b	**Q80K**	V31M, **Y93H**
	M13594-47653-141305	3a	(none)	**A30G**, **Y93H**
	M14868-12641-1301	3a	**Y56H/N**, **Q80K/R**, **Q168R**	P29Q, A30K, **P58T**, **Y93H**
	M14868-32999-7003	3a	**Q80K**, L132I	**L31F**, **Y93H**^{Enr}
	M14868-40525-3409	3a	L132I	Y93H
	M14868-42362-1909	3a	**V107I**^{Enr}	A30K, **Y93H**
	M14868-43362-6801	3a	**Y56H/N**, A166S, **Q168L**	**P29Q**, A30K, **P58Q/T**, **Y93H**
GLE/PIB 16W	M14868-40537-6119	3a	(none)	**L31F**, **Y93H**^{Enr}
	M14868-43362-6809	3a	**A156G**, A166S	**A30K**, **Y93H**^{Enr}
	M14868-45366-4806	3a	Q80K	**M28G**
	M14868-47500-2809	3a	**Y56H**, **Q168R**	A30K, **Y93H**

Collectively, these results indicate that in GT3 infected subjects, baseline NS5A A30K was associated with reduced GLE/PIB efficacy, and treatment failure with GLE/PIB is associated with the emergence of resistance-associated substitutions in both drug targets. The clinical impact of GLE/PIB-based treatment failure is unknown as no data are currently available on the re-treatment of patients who have failed GLE/PIB treatment, although presumably GLE/PIB failure would not impact the anti-HCV activity of sofosbuvir.

Technical Comment about GT3 Resistance Analyses

During the review it was noted that for certain GT3 baseline resistance analyses my analysis results did not match 100% with the sponsor's results. An information request for clarification was communicated to the sponsor on 2/13/2017, and the sponsor responded in SDN 9. According to the sponsor's response, the discrepancies between our analyses were due to different definitions of "baseline" being applied between the sponsor's integrated resistance *dataset* and the sponsor's integrated resistance *report*. Apparently for 12 subjects, all from M14-868 (SURVEYOR-2), "baseline" resistance analysis samples had a Day 1 collection *time* reported after the initiation of dosing, and therefore were considered post-baseline isolates for the purpose of populating the sponsor's analysis tables in the integrated resistance report, while still considered baseline isolates in the resistance dataset. The sponsor updated relevant tables in the integrated resistance report to ensure consistency with the resistance dataset. A slight delay in timing that resulted in resistance sample collection following initiation of dosing on the same day, if indeed true, is unlikely to impact conclusions from the baseline resistance analyses. Eight of the 12 subjects were infected with HCV GT3 (remainder were infected with GT2 or GT4), and among the 8 GT3 infected subjects, 2 had an NS3 A166S polymorphism, and 1 had NS5A A30S and Y93H polymorphisms. All 12 subjects achieved SVR12. See SDN 9 for details.

GLE/PIB 8-Week versus 12-Week Duration for Noncirrhotic, Treatment-naïve, HCV GT3 Infected Patients

The decision to recommend GLE/PIB for 8 weeks versus 12 weeks was a major review issue due to the ~2% higher relapse rate observed with the 8-week duration, as well as treatment-emergent resistance selection in GLE/PIB failures. Further statistical and virology analyses did not identify a specific subgroup that clearly benefited from a 12-week versus 8-week treatment duration, with the possible exception of subjects with the baseline NS5A A30K polymorphism as noted above (based on A30K detection in only 3 relapsers w/8-week duration). During the review the sponsor reported SVR12 results from an additional 21 HCV GT3 infected subjects with HIV-1 coinfection who received GLE/PIB for 8 weeks in an ongoing trial (EXPEDITION-2), and all 21 subjects achieved SVR12. The sponsor also reported that 5 HCV GT3 infected subjects who failed treatment with GLE/PIB in Phase 2 trials were successfully re-treated with SOF-based regimens outside of GLE/PIB clinical trials. Following numerous internal discussions, the review team concluded, and this reviewer agrees, that GLE/PIB for 8 weeks for treatment-naïve, noncirrhotic HCV GT3 infected patients is well supported based on the high SVR12 rate and low relapse rate (<3%) observed in clinical trials, with a reasonable sample size (n=186 in NDA who received GLE/PIB for 8 weeks). For additional details on this review issue, see the internal Midcycle-related discussion slides in Appendix I.

5.5 HCV GT4-6 Resistance Analyses

HCV GT4 Infected Subjects

As noted above in the individual trial analyses, several HCV GT4 subtypes were represented in Phase 2b/3 trials, although subtypes 4a and 4d were predominant, consistent with previous DAA trials. Table 50 (FDA analysis) provides a pooled summary HCV GT4 subtypes represented in clinical trials. No HCV GT4 infected subjects experienced virologic failure across Phase 2b/3 trials of GLE/PIB. Among HCV GT4 infected subjects enrolled at U.S. study sites, 40/47 (85%) had a subtype 4a infection.

Table 50. Pooled summary of HCV GT4 subtypes represented in Phase 2b/3 trials (derived subtype result, prioritizing NS3/NS5A phylogenetic analysis results; non-VF-censored).

HCV Genotype	Cirrhosis?	Regimen	HCV Subtype	N
GT4 (n=174, non-VF-censored, 100% SVR12)	N	GLE/PIB 8W	4	2
			4a	25
			4a/c/d	3
			4d	7
			4f	1
			4k	1
			4m	1
			4v	3
		GLE/PIB 12W	4	2
			4a	44
			4a/4c/4d	1
			4c	1
			4d	40
			4f	1
			4g	2
			4g/4k	1
			4h	1
			4k	7
			4m	1
			4o	2
			4r	7
			4t	1
	Y	GLE/PIB 12W	4	1
			4a	9
			4d	4
			4k	1
			4n	1
			4o	2
			4q	2

Table 51 and Table 52 (FDA analyses) summarize the baseline amino acid polymorphisms detected at resistance-associated positions in NS3 and NS5A, respectively, relative to GT4 subtype-specific references. Considering the key NS3 resistance-associated positions (155, 156, 168), only 1.2% (2/163) HCV GT4 infected subjects with available data had virus with a polymorphism at a key NS3 resistance-associated position (both D168E), while 50% (80/160) of subjects had virus with an NS5A resistance-associated polymorphism.

Table 51. NS3 polymorphisms observed according to GT4 subtype in Phase 2b/3 trials, excluding MAGELLAN-1 (15% NGS cutoff, non-VF-censored dataset). Subtype-specific reference strains were used for resistance analyses.

Subtype	N w/ data	Ref Strain	NS3 Position, Reference Strain Amino Acids, and Polymorphisms Detected												
			36	43	54	55	56	107	122	132	155	156	158	168	170
4a	77	ED43	L	F	T	V	Y	V	T	I	R	A	V	D	V
					S (n=4)			I (n=4)	A (n=1), N (n=1)	L (n=3)					I (n=8)
4c	1	QC381	L	F	T	V	Y	V	T	I	R	A	V	D	V
										L (n=1)					
4d	51	QC382	L	F	T	V	Y	V	T	I	R	A	V	D	V
								I (n=1)	N (n=2)					E (n=1)	
4f	2	IFBT88	L	F	T	V	Y	V	T	I	R	A	V	D	V
							F (n=1)		N (n=1)						
4g	2	QC193	L	F	T	V	Y	V	T	I	R	A	V	D	V
										L (n=1)					
4k	9	QC383	L	F	T	V	Y	V	T	I	R	A	V	D	V
								I (n=1)						E (n=1)	
4m	2	QC249	L	F	T	V	Y	V	S	I	R	A	V	D	I
															V (n=2)
4n	1	QC97	L	F	T	V	Y	V	T	I	R	A	V	D	V
4o	4	QC93	L	F	T	V	Y	V	T	I	R	A	V	D	V
4q	2	QC262	L	F	T	V	Y	V	N	L	R	A	V	D	V
								I (n=1)		I (n=2)					
4r	7	QC384	L	F	T	V	Y	V	T	I	R	A	V	D	V
								I (n=1)							
4t	1	QC155	L	F	T	V	Y	V	T	I	R	A	V	D	V
4v	3	CYHCV048	L	F	T	V	Y	V	S	I	R	A	V	D	V
									N (n=2)	L (n=1)					I (n=1)

Table 52. NS5A polymorphisms observed according to GT4 subtype in Phase 2b/3 trials, excluding MAGELLAN-1 (15% NGS cutoff, non-VF-censored dataset). Subtype-specific reference strains were used for resistance analyses.

Subtype	N w/ data	Ref Strain	NS5A Position, Reference Strain Amino Acids, and Polymorphisms Detected								
			24	28	29	30	31	32	58	92	93
4a	77	ED43	K	L	P	L	M	P	P	A	Y
				M (n=10)		R (n=8)			L (n=1), S (n=2), T (n=2)		
4c	1	QC381	K	L	P	R	M	P	P	A	Y
4d	51	QC382	K	L	P	R	M	P	T	A	Y
							L (n=1), V (n=1)		A (n=2), L (n=1), P (n=40)		
4f	2	IFBT88	K	L	P	Q	M	P	P	A	Y
						R (n=2)	L (n=1)		T (n=1)		
4g	1	QC193	K	L	P	L	M	P	P	A	H
						C (n=1)	L (n=1)				Y (n=1)
4k	8	QC383	K	L	P	R	M	P	P	A	Y
							L (n=7)				
4m	2	QC249	K	L	P	S	M	P	P	A	Y
4n	1	QC97	K	L	P	R	M	P	T	A	Y
4o	3	QC93	K	M	P	T	M	P	P	A	Y
						A (n=1)	L (n=1)				
4q	2	QC262	K	L	P	R	M	P	P	A	Y
4r	7	QC384	K	I	P	R	L	P	P	A	Y
				M (n=3), V (n=3)		H (n=1)					H (n=1)
4t	1	QC155	K	L	P	R	M	P	P	A	Y
4v	3	CYHCV048	K	L	P	R	M	P	P	A	Y

HCV GT5 Infected Subjects

Among 32 HCV GT5 infected subjects in Phase 2b/3 trials, all subjects had a subtype 5a infection, which is the only confirmed subtype of genotype 5 (https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/35/table-1-confirmed-hcv-genotypessubtypes-may-2015). Table 53 and Table 54 (FDA analyses) summarize the baseline amino acid polymorphisms detected at resistance-associated positions in NS3 and NS5A, respectively, for 31 subjects with available data. No HCV GT5 subjects experienced virologic failure across Phase 2b/3 trials of GLE/PIB. Only one HCV GT5 infected subject was enrolled at a U.S. study site.

Table 53. NS3 polymorphisms observed in subjects with HCV GT5a infection in Phase 2b/3 trials (15% NGS cutoff, non-VF-censored dataset).

N w/ Data	Ref Strain	NS3 Position and Reference AAs												
		36	43	54	55	56	107	122	132	155	156	158	168	170
31	SA13	L	F	T	V	F	V	T	I	R	A	V	D	I
					I (n=1)	Y (n=1)	I (n=1)	A (n=10), G (n=2), N (n=2), S (n=2)				M (n=1)	E (n=13)	T (n=1), V (n=5)

Table 54. NS5A polymorphisms observed in subjects with HCV GT5a infection in Phase 2b/3 trials (15% NGS cutoff, non-VF-censored dataset).

N w/ Data	Ref Strain	NS5A Position and Reference AAs								
		24	28	29	30	31	32	58	92	93
31	SA13	Q	L	P	Q	L	P	P	A	T
					R (n=2)	F (n=1)			S (n=1)	

Of particular interest, an NS3 D168E polymorphism was detected in 42% (13/31) of HCV GT5a infected subjects with available NS3/4A sequence analysis data (note that the single U.S. subject did not have D168E). Among the 4 HCV GT5 infected subjects who received the label-proposed regimens (2 noncirrhotic treated with GLE/PIB for 8 weeks, 2 cirrhotic treated with GLE/PIB for 12 weeks), only one subject (M14172-94192-403502, cirrhotic) had the D168E polymorphism. The other 12 subjects with D168E were all noncirrhotic and received the longer, non-proposed 12-week duration of GLE/PIB and achieved SVR12. Thus, all 13 HCV GT5 infected subjects with the reported D168E polymorphism received the 12-week duration. Furthermore, resistance-associated polymorphisms in NS5A were detected in 13% (4/31) of subjects, all of whom received GLE/PIB for 12 weeks.

Various amino acid substitutions at NS3 position D168, alone or in combination with other NS3 RASs, can reduce GLE activity in multiple HCV genotypes/subtypes (Appendix A). No data were included in the initial NDA submission to address the impact of the NS3 D168E polymorphism on the activity of GLE against HCV GT5a in cell culture, although as noted in Section 2.2, late in the review cycle (SDN 31) the sponsor commented briefly that introduction of an NS3 D168E substitution in a GT5a replicon caused a <5-fold reduction in GLE activity. While these data are encouraging and indicate that GLE maintains reasonable activity against HCV GT5a with a D168E polymorphism, it remains unclear if the D168E polymorphism affects durability of response. In the MAGELLAN-1 trial that included NS3/4A PI- or NS5A inhibitor-experienced subjects, virologic failure was associated with the detection of certain baseline NS3 or NS5A RASs, which in many cases did not confer clear resistance to GLE or PIB when engineered into HCV replicons as single amino acid substitutions (see Section 6.2 for details). Ultimately SVR data are needed to confirm whether a baseline amino acid polymorphism has a significant effect on treatment outcome.

In this reviewer's opinion, at the present time the sponsor does not have sufficient evidence of treatment efficacy (i.e., SVR) to recommend the 8-week treatment duration for noncirrhotic subjects with HCV GT5. Favorable efficacy results with the 8-week duration in a small number of HCV GT5 infected subjects could support the use of this duration; however, only 2 HCV GT5 infected subjects received GLE/PIB for 8 weeks, and these subjects were not representative of the larger GT5 population. One of these subjects did not have the NS3 D168E polymorphism, and the other subject did not have available NS3 sequence analysis data (Table 55, FDA analysis). Furthermore, both subjects had low baseline HCV RNA levels; these subjects had the second and third lowest HCV RNA levels of all 32 HCV GT5 infected subjects in the dataset. Use of the longer, 12-week treatment duration for this population is better supported with an SVR12 rate of 28/28 (100%) overall, including 12/12 (100%) for subjects with the NS3 D168E polymorphism. The small sample size for HCV GT5 infected subjects with cirrhosis (n=2) is not ideal, but a 12-week treatment duration is reasonable for

this population based on the totality of data across different genotypes, and given the impracticality of enrolling large numbers of subjects with GT5 and cirrhosis.

Table 55. HCV GT5a infected subjects who received GLE/PIB for 8 weeks (n=2).

USUBJID	HCV Subtype	Tx History	Regimen	BL HCV RNA (IU/mL)	Cirrhosis?	NS3 D168E?	SVR12
M14868-16471-7730	5a	Naïve	GLE/PIB 8W	177,000	N	N	Y
M14868-29080-8827	5a	Naïve	GLE/PIB 8W	161,000	N	(No Data)	Y

At the time of completion of this review, the review team agreed that the sponsor does not have sufficient evidence to support the 8-week GLE/PIB duration for noncirrhotic patients with HCV GT5 infection. However, GLE/PIB for 12 weeks is supported in this population based on a 28/28 (100%) SVR12 rate across clinical trials. For additional details on this review issue, see the internal Midcycle-related discussion slides in Appendix I.

HCV GT6 Infected Subjects

Similar to HCV GT4, HCV GT6 is extremely genetically diverse with at least 28 reported subtypes (https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/35/table-1-confirmed-hcv-genotypessubtypes-may-2015). Table 56 (FDA analysis) provides a pooled summary HCV GT6 subtypes represented in clinical trials. No HCV GT6 infected subjects experienced virologic failure across Phase 2b/3 trials of GLE/PIB. Only 5 HCV GT6 infected subjects enrolled at a U.S. study site; all 5 subjects had a subtype 6e infection.

Table 56. Pooled summary of HCV GT6 subtypes represented in Phase 2b/3 trials (derived subtype result, prioritizing NS3/NS5A phylogenetic analysis results; non-VF-censored).

HCV Genotype	Cirrhosis?	Regimen	HCV Subtype	N
GT6 (n=47, non-VF-censored, 100% SVR12)	N	GLE/PIB 8W	6a	3
			6a/b	2
			6e	3
			6l	1
		GLE/PIB 12W	6	1
			6a	7
			6c-l	6
			6e	8
			6h	2
			6p	2
			6q	2
			6r	3
	Y	GLE/PIB 12W	6a	1
			6e	5
			6t	1

Table 57 and Table 58 (FDA analyses) summarize the baseline amino acid polymorphisms detected at resistance-associated positions in NS3 and NS5A, respectively, relative to GT6 subtype-specific references. Considering the key NS3 resistance-associated positions (155, 156, 168), only 3% (1/34) of HCV GT6 infected subjects with available data had virus with a polymorphism at a key NS3 resistance-associated position (D168E), while 54% (20/37) of subjects had virus with an NS5A resistance-associated polymorphism (relative to a subtype-specific reference).

Table 57. NS3 polymorphisms observed according to GT6 subtype in Phase 2b/3 trials (15% NGS cutoff, non-VF-censored dataset). Subtype-specific reference strains were used for resistance analyses.

Subtype	N w/ data	Ref Strain	NS3 Position, Reference Strain Amino Acids, and Polymorphisms Detected												
			36	43	54	55	56	107	122	132	155	156	158	168	170
6a	11	EUHK2	V	F	T	V	Y	V	S	I	R	A	V	D	I
								I (n=1)	N (n=10)				I (n=1)	E (n=1)	
6e	15	GX004	V	F	T	V	Y	V	T	L	R	A	V	D	V
			L (n=2)						N (n=1)						
6h	1	VN004	V	F	T	V	Y	V	N	I	R	A	V	D	A
															V (n=1)
6p	2	QC216	V	F	T	V	Y	V	T	L	R	A	V	D	V
			I (n=1)					I (n=1)							
6q	2	QC99	I	F	T	V	Y	V	T	L	R	A	V	D	V
6r	3	QC245	V	F	T	V	F	V	T	L	R	A	V	D	V
								I (n=1)		V (n=1)					

Table 58. NS5A polymorphisms observed according to GT6 subtype in Phase 2b/3 trials (15% NGS cutoff, non-VF-censored dataset). Subtype-specific reference strains were used for resistance analyses.

Subtype	N w/ data	Ref Strain	NS5A Position, Reference Strain Amino Acids, and Polymorphisms Detected								
			24	28	29	30	31	32	58	92	93
6a	11	EUHK2	Q	F	P	R	L	P	T	A	T
			K (n=1), R (n=1)	L (n=6)							
6e	16	GX004	K	V	P	S	L	P	P	A	T
			R (n=2)	M (n=6)			I (n=1)		S (n=3)		S (n=2)
6h	1	VN004	K	V	P	A	L	P	P	A	T
6l	1	537796	K	V	P	A	L	P	P	A	T
6p	2	QC216	K	V	P	S	L	P	P	A	T
				M (n=1)							
6q	2	QC99	K	V	P	S	L	P	P	A	T
6r	3	QC245	K	G	P	A	L	P	P	A	T
			R (n=1)	A (n=2), T (n=2)							
6t	1	D49	K	V	P	S	L	P	G	A	T

A key review question related to HCV GT6 was whether the sponsor has sufficient evidence to support recommending GLE/PIB for the short, 8-week duration for noncirrhotic patients with HCV GT6 infection. Of the 9 HCV GT6 infected subjects who achieved SVR12 with GLE/PIB for 8 weeks across Phase 2b/3 trials (all were enrolled in SURVEYOR-2), 7 had available baseline NS5A sequence analysis data and 6 had available baseline NS3 sequence analysis data. Polymorphisms (relative to subtype-specific references) observed in

these subjects at resistance-associated positions are summarized in Table 59 (FDA analysis). None of these subjects had any within-subtype resistance-associated polymorphisms of significant concern (e.g., at NS3 positions 156 or 168, or NS5A positions 30 or 93). However, it is important to note that the "wild-type" amino acid sequences at multiple key positions in NS5A, including at positions 28, 30 and 93, vary across different subtypes within GT6, and also between GT6 and other HCV genotypes. One HCV GT6 infected subject (M13583-49101-201403) had an NS3 D168E polymorphism that may reduce GLE activity; however, this subject was noncirrhotic and received GLE/PIB for 12 weeks. The two subjects without any NS3 or NS5A baseline sequence analysis data had an indeterminate HCV subtype (a/b) and were treatment-experienced. Based on the limited SVR data, it is not possible to draw any firm conclusions regarding the potential impact of HCV GT6 genetic diversity or baseline polymorphisms on the efficacy of an 8-week treatment duration of GLE/PIB.

Table 59. Baseline polymorphisms (relative to subtype-specific references) detected at potential resistance-associated positions for HCV GT6 infected subjects who achieved SVR12 with GLE/PIB for 8 weeks (15% NGS cutoff).

USUBJID	Tx Experience	HCV Subtype	Ref.	NS3 Polymorphisms	NS5A Polymorphisms
M14868-29080-8829	Naïve	6a	EUHK2	0	0
M14868-40622-2710	Naïve	6a	EUHK2	S122N, V158I	Q24R
M14868-47500-2814	Naïve	6a	EUHK2	S122N	F28L
M14868-09818-9322	Naïve	6e	GX004	T122N	0
M14868-16471-7727	Naïve	6e	GX004	0	V28M, P58S
M14868-16471-7732	Naïve	6e	GX004	0	P58S
M14868-40621-9615	Naïve	6l	537796	No Data	0
M14868-40621-9614	IFN- or P/R-exp.	6a/b	No Data		
M14868-47500-2812	IFN- or P/R-exp.	6a/b	No Data		

At the time of completion of this review, the review team concluded that, due to a limited sample size (n=9, non-VF-censored), coupled with the extensive diversity of GT6 subtypes that includes amino acid variability at multiple NS5A resistance-associated positions, the sponsor does not have sufficient evidence to support the 8-week GLE/PIB duration for noncirrhotic patients with HCV GT6 infection. Late in the review cycle (SDNs 22 and 31) the sponsor commented that the one GT6 subject in SURVEYOR-2 who did not achieve SVR12 with GLE/PIB for 8 weeks (lost to follow-up) subsequently returned to the study site and was found to have achieved SVR12, and 3 other HCV GT6 infected subjects with HIV-1 coinfection who received GLE/PIB for 8 weeks in the ongoing EXPEDITION-2 trial achieved SVR12. Thus, 13/13 (100%) HCV GT6 infected subjects have achieved SVR12 with GLE/PIB for 8 weeks. Nevertheless, GLE/PIB for 12 weeks is still better supported based on a 31/31 (100%) SVR12 rate across trials. Furthermore, the other 13 subjects who achieved SVR12 with the 8-week duration theoretically would have achieved SVR12 with a 12-week duration, and thus the 12-week regimen is effectively supported by a 100% SVR12 rate in 44 noncirrhotic subjects, plus 7 other subjects with cirrhosis. For additional details on this review issue, see the internal Midcycle-related discussion slides in Appendix I.

5.6 Other Exploratory Resistance Analyses

NS3 Position Q/P89

As noted in Section 2.2, substitutions at NS3 amino acid position Q/P89 in combination with NS3 A156 substitutions enhanced HCV replication capacity and/or resistance to GLE in cell culture. However, amino acid variability at this position was not clinically associated with treatment failure. Across the full Phase 2b/3 resistance dataset (to-be-marketed doses, non-VF-censored) for NS3/4A PI-naïve and NS5A inhibitor-naïve populations, 136 subjects had a polymorphism reported at position NS3 89 (Table 60), of whom only 1 did not

achieve SVR12 (P89S, Subject M13594-09818-162318, GT3, GLE/PIB 8W; Subject also had NS5A A30K polymorphism). No virologic failure subjects in the Phase 2b/3 dataset had treatment-emergent substitutions at NS3 position 89.

Table 60. Number of subjects with baseline polymorphisms (≥15% NGS cutoff) at NS3 position 89.

HCV Subtype	NS3 Position 89 PM (≥15% NGS)	Number of Subjects
1	P89Q	1
1a	Q89A/S	1
	Q89H	6
	Q89N	1
	Q89P	5
	Q89P/H	1
	Q89Q/H	1
	Q89S	1
	Q89Y	2
1b	P89A	8
	P89P/A	1
	P89P/S	19
	P89P/S/A	1
	P89Q	1
	P89S	62
	P89S/A	1
	P89V	1
2l	P89S	1
3a	P89A	1
	P89P/Q	1
	P89P/S	5
	P89S	10
4d	P89S	1
5a	P89S	1
6h	A89P	1
6q	T89A	1
	T89P	1

Unbiased Treatment-Emergent Resistance Analysis

An additional exploratory analysis was conducted to identify any potentially novel treatment-emergent resistance-associated substitutions in NS3/4A or NS5A. An unbiased analysis of all amino acid positions was conducted for 24 subjects who experienced virologic failure (18 GT3, 2 GT1, 2 GT2). Since treatment-emergent substitutions at known resistance-associated positions typically predominated (often >99% of amino acids detected) when they were detected, only those substitutions that emerged at a frequency ≥15% were considered for further analysis to reduce the likelihood of false-positive detection of potentially novel resistance-associated substitutions.

The only potentially novel position where a substitution emerged to a frequency ≥15% in ≥1 subject was at NS3 position I366, which is in the NS3 helicase domain (i.e., outside of the protease domain that spans amino acids 1-181). Two subjects (M13594-47653-141305, M13594-53762-140310, both subtype 3a) had treatment-emergent I366V. Subject 141305 had NS3 I366V emerge to a 35% level, as well as treatment-emergent NS5A RASs A30G (33%) and Y93H (>99%), but no known NS3 RASs were detected. Subject 140310 had NS3 I366V emerge to a >99% level, and also had treatment-emergent NS5A Y93H (>99%), but again no known NS3 RASs emerged (only a minor A166Y substitution, not clearly associated with resistance). Position I366

was relatively conserved among GT3a subjects, with only 23/742 (3%) having an I366V polymorphism (≥15% NGS or population sequencing), two of whom experienced virologic failure with a GLE/PIB regimen. The detection of NS5A RASs but no clear NS3 RASs in the 2 subjects with treatment-emergent NS3 I366V indicates that I366V could be a novel NS3 RAS, and therefore the sponsor should conduct phenotypic analyses to assess if this substitution impacts GLE anti-HCV activity. Although not common, treatment-emergent substitutions have been detected in the NS3 helicase domain following treatment failure with certain other NS3/4A PI-containing regimens (e.g., E357K, Viekira Pak™ label).

6. M15-410/MAGELLAN-1 (NS3/4A PI or NS5A-EXP) EFFICACY AND RESISTANCE ANALYSES

6.1 MAGELLAN-1 Efficacy Analyses

Title
M15-410, "A Randomized, Open-Label, Multicenter Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of Co-Administration of ABT-493 and ABT-530 (or ABT-493/ABT-530) with and without Ribavirin in Adults with Chronic Hepatitis C Virus (HCV) Infection Who Failed a Prior Direct-Acting Antiviral Agent (DAA)-Containing Therapy (MAGELLAN-1)"

Summary of Design and Study Population
Clinical trial M15-410 (MAGELLAN-1) was an expanded Phase 2, randomized, open-label, multicenter study to evaluate GLE/PIB dosed with or without RBV in subjects with chronic HCV GT1 or GT4-6 infection who failed a prior anti-HCV DAA-containing regimen.

The study was conducted sequentially in two Parts (Figure 5, CSR pg. 94). Individual DAA formulations were used in Part 1, and the co-formulated GLE/PIB tablets (3x 100 mg/40 mg) were used in Part 2. In both Parts, subjects were to be naïve to GLE and PIB. Positive test results for HBV surface antigen or anti-HIV antibody were also exclusionary.

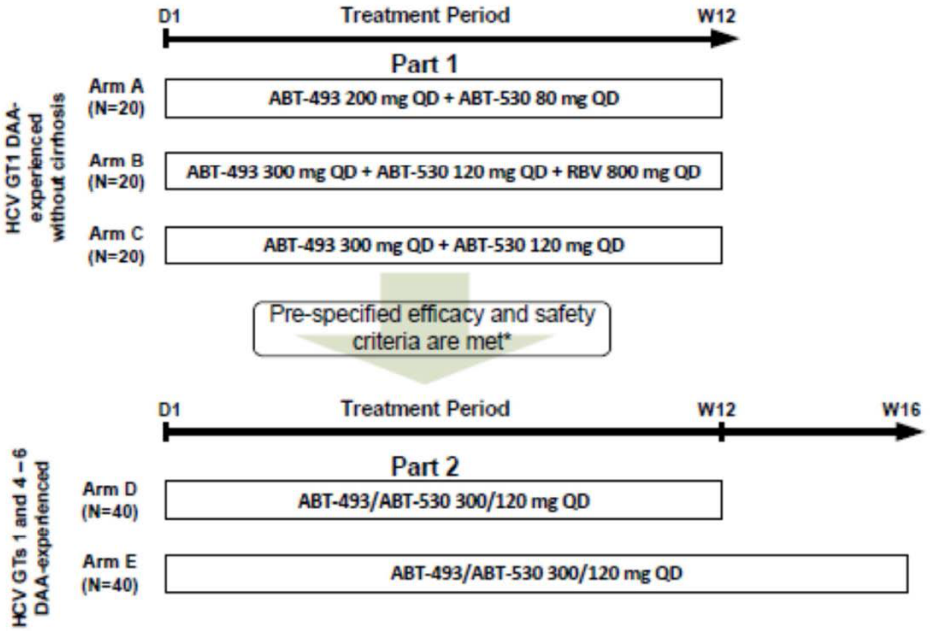


Figure 5. Study design schematic for clinical trial M15-410 (MAGELLAN-1). ABT-493=glecaprevir (GLE), ABT-530=pibrentasvir (PIB).

In Part 1, approximately 50 DAA-experienced, noncirrhotic subjects with HCV GT1 infection were to be enrolled and randomized in a 1:1:1 ratio to Arm A, B or C. During the conduct of the trial, enrollment in Arm A was stopped with Protocol Amendment 3 based upon the sponsor's decision not to pursue development of the doses in Arm A (GLE 200 mg + PIB 80 mg). Subjects were subsequently randomized in a 1:1 ratio to Arms B or C. Randomization was stratified by HCV GT1 subtype and prior DAA class experience (NS5A inhibitor ± NS3/4A PI, NS3/4A PI only, or other DAA class experience {e.g., SOF}).

In Part 2, approximately 80 subjects with HCV GT1, GT4, GT5 or GT6 infection were to be enrolled. Subjects could be noncirrhotic or could have compensated cirrhosis. Subjects were to be randomized in a 1:1 ratio to Arm D or E, in which GLE/PIB (300 mg/120 mg) was administered for either 12 or 16 weeks, respectively. Randomization was stratified by HCV GT (GT1 or GT4-6) and by prior DAA class experience (NS5A inhibitor ± NS3/4A PI, or NS3/4A PI only). For Part 2, prior DAA experience was limited to specific DAAs. NS5A inhibitor experience could have included regimens containing daclatasvir (DCV), ledipasvir (LDV) or ombitasvir (OBV). NS3/4A PI experience could have included regimens containing paritaprevir/ritonavir (PTV/r), simeprevir (SMV), telaprevir (TVR) or boceprevir (BOC).

FDA Clinical Virology Analysis of Efficacy

A total of 50 subjects enrolled in Part 1: 6 in Arm A, 22 in Arm B and 22 in Arm C. A total of 91 subjects enrolled in Part 2: 44 in Arm D and 47 in Arm E. Per protocol, all subjects in Part 1 were infected with HCV GT1. Among subjects in Part 2, 27/91(30%) had compensated cirrhosis. Although other HCV genotypes were eligible for Part 2, 87/91 (96%) were infected with HCV GT1 and the remainder (4/91, 4%) were infected with HCV GT4. Unless indicated otherwise, the following efficacy and resistance analyses focused primarily on HCV GT1 infected subjects, as they contributed to all but 4 of the subjects enrolled.

As shown in Figure 6 (FDA analysis), specific DAA treatment experience varied extensively across all patients and also according to prior DAA class treatment history. In the NS5A inhibitor-naïve subgroup, most PI experience was with earlier generation PIs such as BOC, SMV and TVR, which is not surprising as these PIs are not approved for use in combination with an NS5A inhibitor. In contrast, for NS3/4A PI- and NS5A inhibitor-experienced subjects, the most commonly used PI was PTV/r, which is only approved in a fixed-dose combination with an NS5A inhibitor (OBV). This may have impacted the specific NS3 RASs represented these subgroups as the resistance pathways for PTV/r (primarily NS3 D168 and also some Y56 substitutions) (Viekira Pak™ label) differ somewhat from the resistance pathways with earlier NS3/4A PIs, particularly BOC and TVR (primarily R155K and limited D168 substitutions) (Victrelis™ label, Incivek™ label, respectively). Similarly, the NS5A inhibitor experience varied according to whether subjects also had prior NS3/4A PI experience, again not surprising based on the clinical use of these DAAs, although this imbalance is not a significant concern as the resistance pathways for LDV, DCV and OBV are similar (Harvoni™ label, Daklinza™ label, Viekira Pak™ label, respectively).

No subjects had prior experience with the more recently approved NS5A inhibitor plus NS3/4A PI combination of elbasvir and grazoprevir (EBR/GZR, Zepatier™ label). The NS3 resistance pathways for GZR are similar to those for PTV/r, and the NS5A resistance pathways for EBR are similar to those of other NS5A inhibitors. In addition, no subjects received the recently approved NS5A inhibitor, velpatasvir (VEL), which is dosed in combination with SOF (Epclusa™ label). Due to limited cases of SOF/VEL virologic failure in clinical trials, the most common VEL treatment-emergent resistance pathways in HCV GT1 are unknown, but at least based on nonclinical studies VEL has overlapping resistance mechanisms with other NS5A inhibitors.

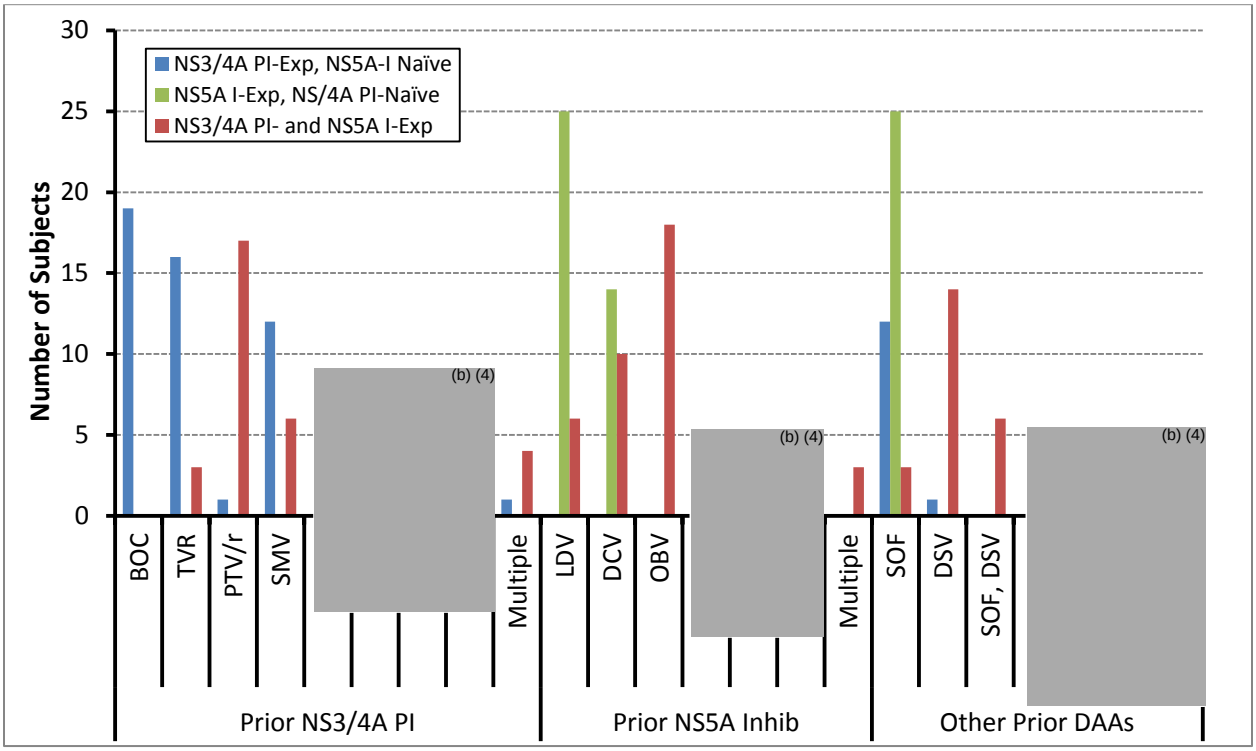


Figure 6. Specific DAA treatment history according to prior DAA class treatment history (GT1, non-VF-censored dataset). NS3/4A PI abbreviations: BOC, boceprevir; TVR, telaprevir; PTV/r, paritaprevir/ritonavir; SMV, simeprevir; (b) (4) NS5A inhibitor abbreviations: LDV, ledipasvir; DCV, daclatasvir; OBV, ombitasvir; (b) (4) Other DAA classes: SOF, sofosbuvir (uridine nucleotide analogue NS5B polymerase inhibitor); DSV, dasabuvir (non-nucleoside NS5B palm polymerase inhibitor); (b) (4) *Indicates investigational DAA.

Overall SVR12 and virologic failure results are summarized in Table 61 (FDA analysis). SVR12 rates ranged from 86-100% across treatment arms.

Table 61. Overall SVR12 (ITT) and virologic failure results in clinical trial MAGELLAN-1.

Trial Part	Arm: Regimen	HCV GT	SVR12	Virologic Failure			Non-VF
				Any VF	On-Tx VF	Relapse	
Part 1	A: GLE+PIB (200+80) 12W	GT1	100% (6/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
	B: GLE+PIB+RBV 12W	GT1	95% (21/22)	5% (1/22)	0% (0/22)	5% (1/22)	0% (0/22)
	C: GLE+PIB 12W	GT1	86% (19/22)	5% (1/22)	5% (1/22)	0% (0/22)	9% (2/22)[1]
Part 2	D: GLE/PIB 12W	GT1	88% (38/43)	12% (5/43)	2% (1/43)	9% (4/43)	0% (0/43)
		GT4	1/1	0/1	0/1	0/1	0/1
	E: GLE/PIB 16W	GT1	91% (40/44)	9% (4/44)	9% (4/44)	0% (0/44)	0% (0/44)
		GT4	100% (3/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)

[1]Non-VFs (n=2 in Arm C) were due to missing SVR12 data: Subject 1301 (GT1a, NS3/4A PI-experienced, NS5A inhibitor-naïve), and Subject 2204 (GT1a, NS3/4A PI-experienced, NS5A inhibitor-naïve). Both subjects had HCV RNA Target Not Detected at the last available Post-Treatment visit (Post-Treatment Day 56 for both).

As shown in Figure 7 (FDA analysis), no HCV GT1 infected, NS3/4A PI-experienced, NS5A inhibitor-naïve subjects experienced virologic failure in any of the treatment arms. All 11 subjects who experienced virologic failure had treatment experience with an NS5A inhibitor-containing regimen.

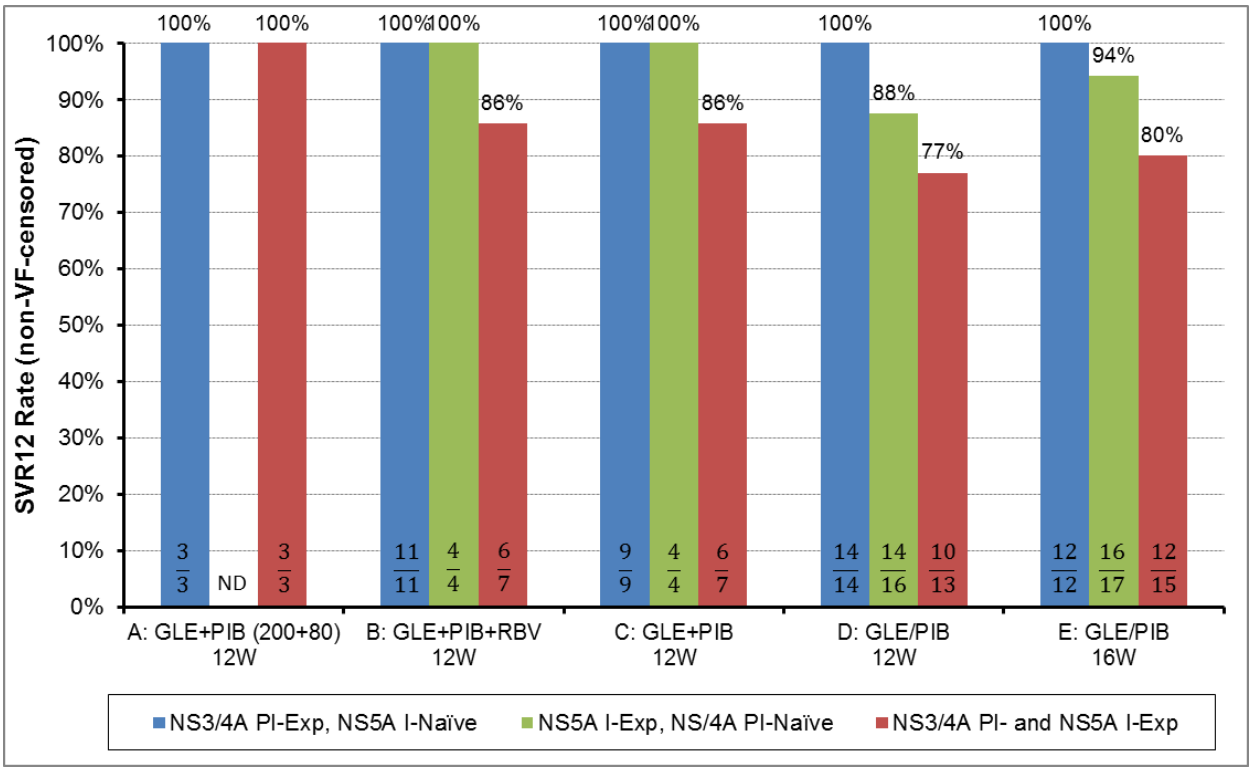


Figure 7. SVR12 (non-VF-censored) results for HCV GT1 infected subjects in MAGELLAN-1 according to prior DAA class experience.

Efficacy results according to HCV subtype 1a/1b are summarized in Table 62 (FDA analysis). Across all treatment arms, virologic failure occurred in 10/107 (9%) and 1/26 (4%) subjects with HCV GT1a or GT1b, respectively (non-VF-censored population). Two subjects had rare HCV GT1 subtypes 1c or 1e, both of whom enrolled in Arm E, were NS5A inhibitor-experienced/NS3/4A PI-naïve, and achieved SVR12.

Table 62. SVR12 (non-VF-censored) results for HCV GT1a and GT1b infected subjects in MAGELLAN-1.

Arm	HCV Subtype	SVR12	Virologic Failure	On-Tx VF	Relapse
A: GLE+PIB (200+80) 12W	1a	100% (4/4)	0% (0/4)	0	0
	1b	100% (2/2)	0% (0/2)	0	0
B: GLE+PIB+RBV 12W	1a	95% (19/20)	5% (1/20)	0	1
	1b	100% (2/2)	0% (0/2)	0	0
C: GLE+PIB 12W	1a	94% (15/16)	6% (1/16)	1	0
	1b	100% (4/4)	0% (0/4)	0	0
D: GLE/PIB 12W	1a	89% (31/35)	11% (4/35)	1	3
	1b	88% (7/8)	13% (1/8)	0	1
E: GLE/PIB 16W	1a	88% (28/32)	13% (4/32)	4	0
	1b	100% (10/10)	0% (0/10)	0	0

There was a possible trend of reduced efficacy for those with cirrhosis in Part 2 when accounting for prior DAA class experience, although the numbers of subjects for analysis were too small to draw any firm conclusions on the impact of cirrhosis status (Table 63; FDA analysis).

Table 63. SVR12 results (non-VF-censored) for HCV GT1 infected subjects in MAGELLAN-1 Part 2, according to cirrhosis status.

	All Subjects		NS3/4A PI-Experienced NS5A-I Naïve		NS5A I-Experienced, NS/4A PI-Naïve		NS3/4A PI- and NS5A I-Experienced	
Treatment Arm	**Non-cirr**	**Cirrhotic**	**Non-cirr**	**Cirrhotic**	**Non-cirr**	**Cirrhotic**	**Non-cirr**	**Cirrhotic**
D: GLE/PIB 12W	86% (24/28)	93% (14/15)	100% (7/7)	100% (7/7)	89% (8/9)	86% (6/7)	75% (9/12)	100% (1/1)
E: GLE/PIB 16W	97% (33/34)	70% (7/10)	100% (8/8)	100% (4/4)	100% (14/14)	67% (2/3)	92% (11/12)	33% (1/3)

Because NS5A inhibitor experience, with or without NS3/4A protease inhibitor experience, appeared to be the key factor associated with virologic failure in MAGELLAN-1, subsequent analyses focused on these subgroups separately in order to determine the ideal treatment approach for each subpopulation. These analyses pooled Arms A, C and D since the only differences in treatments administered were the DAA formulations used and the slightly lower GLE and PIB dose levels administered in Arm A (which apparently did not reduce treatment efficacy).

Figure 8 (FDA analysis) provides a pooled summary of SVR12 rates (non-VF-censored) according to GLE/PIB regimen and prior DAA class experience. Table 64 (FDA analysis) provides a breakdown of SVR12 rates (non-VF-censored) according to prior specific DAA regimen experience, and Table 65 (FDA analysis) provides additional details for subjects who previously received multiple prior DAA-containing regimens.

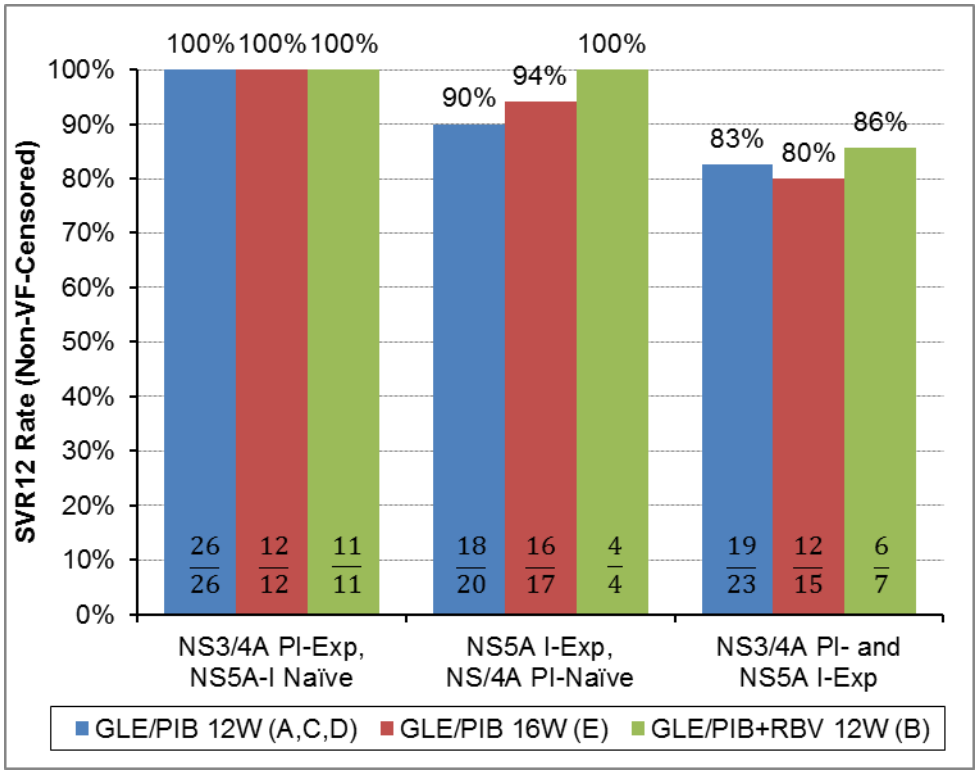


Figure 8. SVR12 (non-VF-censored) results for HCV GT1 infected subjects in MAGELLAN-1, according to pooled DAA regimen group and prior DAA class experience.

Table 64. SVR12 (non-VF-censored) results for HCV GT1 infected subjects according to prior specific DAA regimen experience. See DAA abbreviations in Figure 6 legend; *investigational DAA.

Prior DAA Class Experience	Prior Regimen	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
NS3/4A PI-Exp, NS5A-I Naïve	BOC+P/R	100% (8/8)	100% (6/6)	100% (5/5)	100% (19/19)
	TVR+P/R	100% (9/9)	100% (3/3)	100% (2/2)	100% (14/14)
	SMV+SOF+/-Peg-IFNα	100% (3/3)	100% (2/2)	100% (3/3)	100% (8/8)
	SMV+P/R	100% (2/2)	1/1	1/1	100% (4/4)
	PTV/r+DSV+RBV	1/1	ND	ND	1/1
	Multiple	100% (3/3)	ND	ND	100% (3/3)
	All Prior Regimens	100% (26/26)	100% (12/12)	100% (11/11)	100% (49/49)
NS5A I-Exp, NS/4A PI-Naïve	LDV/SOF+/-RBV	92% (11/12)	89% (8/9)	100% (4/4)	92% (23/25)
	DCV+P/R	88% (7/8)	100% (7/7)	ND	93% (14/15)
	Multiple	ND	1/1	ND	1/1
	All Prior Regimens	90% (18/20)	94% (16/17)	100% (4/4)	93% (38/41)
NS3/4A PI- and NS5A I-Exp	OBV/PTV/r+/-DSV+/-SOF+/-RBV	88% (7/8)	80% (4/5)	100% (2/2)	87% (13/15)
	(b) (4)				
	SMV+DCV+RBV+/-SOF	1/1	1/1	ND	100% (2/2)
	(b) (4)				
	Multiple	67% (2/3)	67% (4/6)	50% (1/2)	64% (7/11)
	All Prior Regimens	83% (19/23)	80% (12/15)	86% (6/7)	82% (37/45)
All Prior Experience	All Prior Regimens	91% (63/69)	91% (40/44)	95% (21/22)	92% (124/135)

Table 65. Prior treatment history and GLE/PIB treatment outcomes for HCV GT1 infected subjects (non-VF-censored) who previously received multiple prior DAA-containing regimens. See DAA abbreviations in Figure 6 legend; *investigational DAA.

Prior DAA Class Exp	USUBJID	Prior Regimens (starting w/most recent)	GT1 Subtype	Cirr?	GLE/PIB Regimen	SVR?
NS3/4A PI-Exp, NS5A-I Naïve	M15410-38627-1302	SOF+P/R, TVR+P/R	1b	N	GLE/PIB 12W (A,C,D)	Y
	M15410-42371-2020	SOF+P/R, TVR+P/R	1a	Y	GLE/PIB 12W (A,C,D)	Y
	M15410-42730-1020	SMV+SOF, TVR+P/R	1a	Y	GLE/PIB 12W (A,C,D)	Y
NS5A I-Exp, NS/4A PI-Naïve	M15410-37759-4527	LDV/SOF+RBV, SOF+P/R	1a	N	GLE/PIB 16W (E)	Y
NS3/4A PI- and NS5A I-Exp	(b) (4)					
	M15410-41723-2624	LDV/SOF, SMV+SOF	1a	N	GLE/PIB 12W (A,C,D)	N
	M15410-51321-4422	OBV/PTV/r+DSV+RBV, BOC+P/R	1a	N	GLE/PIB 12W (A,C,D)	Y
	M15410-12641-1620	OBV/PTV/r+DSV+RBV, LDV/SOF+RBV, SMV+SOF, TVR+P/R	1a	Y	GLE/PIB 16W (E)	N
	(b) (4)					
	M15410-41723-2622	OBV/PTV/r+DSV+RBV, DCV+P/R	1a	N	GLE/PIB 16W (E)	N

	M15410-41723-2623	OBV/PTV/r+DSV+SOF+RBV, TVR+P/R	1a	N	GLE/PIB 16W (E)	Y
	M15410-42730-1024	LDV/SOF, TVR+P/R	1a	N	GLE/PIB 16W (E)	Y
	M15410-92059-3420	LDV/SOF, OBV/PTV/r+DSV+RBV	1a	N	GLE/PIB 16W (E)	Y
	M15410-41723-2601	TVR+P/R, DCV (monotx?)	1a	N	GLE/PIB+RBV 12W (B)	N
	M15410-42371-2003	OBV/PTV/r+DSV+RBV, SMV+SOF+RBV, BOC+P/R	1a	N	GLE/PIB+RBV 12W (B)	Y

For NS3/4A PI-experienced, NS5A inhibitor-naïve subjects, based on the pooled treatment groups and overall SVR12 rates there was no obvious benefit of dosing GLE/PIB for >12 weeks or in combination with RBV.

For NS5A inhibitor-experienced subjects (with or without NS3/4A protease inhibitor experience), again there was no signal that a longer treatment duration (16 weeks) or addition of RBV improved the efficacy of GLE/PIB based on overall SVR12 and VF rates alone. In general, the numbers of subjects who received GLE/PIB + RBV were insufficient to determine if RBV improves treatment efficacy in these subgroups.

On the other hand, if the type and timing of virologic failure are considered, there is some evidence that for NS5A inhibitor-experienced subjects GLE/PIB for 16 weeks was possibly more effective than GLE/PIB for 12 weeks. The primary purpose of a longer treatment duration is to prevent virologic relapse. In the 16-week treatment group, all 4 instances of virologic failure occurred on-treatment at Treatment Week ≤10, and thus would have occurred regardless the assigned GLE/PIB treatment duration. Virologic relapse rates for NS5A inhibitor-experienced, HCV GT1 infected subjects who received GLE/PIB for 12 or 16 weeks were 10% (4/41) and 0% (0/28), respectively (Table 66; FDA analysis).

Table 66. Virologic relapse rate among NS5A inhibitor-experienced, HCV GT1 infected subjects who received GLE/PIB for 12 or 16 weeks. Analysis excludes subjects who experienced virologic breakthrough from the denominator; no NS5A inhibitor experienced subjects failed treatment for non-virologic reasons.

	NS5A Inhibitor Treatment-Experienced Subjects					
	All Subjects		**NS5A I-Exp, NS/4A PI-Naïve**		**NS3/4A PI- and NS5A I-Exp**	
	GLE/PIB 12W (A,C,D)	**GLE/PIB 16W (E)**	**GLE/PIB 12W (A,C,D)**	**GLE/PIB 16W (E)**	**GLE/PIB 12W (A,C,D)**	**GLE/PIB 16W (E)**
Relapse Rate	10% (4/41)	0% (0/28)	5% (1/19)	0% (0/16)	14% (3/22)	0% (0/12)

6.2 MAGELLAN-1 Resistance Analyses

The resistance dataset for MAGELLAN-1 includes data for 139/141 (99%) enrolled subjects. Two subjects, both in Arm E, were not included in the dataset: Subject 5021 (GT1c {rare GT1 subtype}, achieved SVR12) and Subject 3620 (GT4, ambiguous a/c/d subtype, achieved SVR12). In addition one subject (Subject 3723, achieved SVR12) with a subtype 1e infection, another rare GT1 subtype, did not have baseline NS5A sequence analysis data reported. Two additional subjects noted above (Subjects 1301 and 2204) who did not achieve SVR12 for non-virologic reasons were censored in the resistance analyses.

In the analyses described below, both baseline and post-baseline substitutions are referred to as “resistance-associated substitutions” (“RASs”). It should be noted that baseline RASs may include substitutions relative to reference that either pre-existed naturally (i.e. as polymorphisms, or “RAPs”), or were selected by prior DAA

exposure. NGS sensitivity cutoffs of 15% (comparable to Sanger sequencing and current clinical diagnostic methods) and 2% (lowest prevalence changes reported in resistance dataset) were considered in these analyses.

HCV GT1 Baseline Resistance Characteristics

Among subjects infected with HCV GT1a or GT1b in the censored population, 133/133 (100%), including 107 GT1a and 26 GT1b subjects, had baseline data for both drug targets. SVR12 analysis results for these 133 subjects are summarized in Table 67 (FDA analysis). For NS5A inhibitor-experienced subjects, virologic failure rates were 10/69 (14%) for HCV GT1a infected subjects and 1/15 (7%) for HCV GT1b infected subjects, across all arms.

Table 67. SVR12 (non-VF-censored) results for HCV GT1a and GT1b infected subjects in MAGELLAN-1, according to regimen and prior DAA class experience. ND, no data

Regimen Group	Prior DAA Class Exp	SVR12	
		GT1a	GT1b
GLE/PIB 12W (A,C,D)	NS3/4A PI-Exp, NS5A-I Naïve	100% (21/21)	100% (5/5)
	NS5A I-Exp, NS/4A PI-Naïve	87% (13/15)	100% (5/5)
	NS3/4A PI- and NS5A I-Exp	84% (16/19)	75% (3/4)
GLE/PIB 16W (E)	NS3/4A PI-Exp, NS5A-I Naïve	100% (8/8)	100% (4/4)
	NS5A I-Exp, NS/4A PI-Naïve	92% (12/13)	100% (2/2)
	NS3/4A PI- and NS5A I-Exp	73% (8/11)	100% (4/4)
GLE/PIB+RBV 12W (B)	NS3/4A PI-Exp, NS5A-I Naïve	100% (9/9)	100% (2/2)
	NS5A I-Exp, NS/4A PI-Naïve	100% (4/4)	ND
	NS3/4A PI- and NS5A I-Exp	86% (6/7)	ND

The following known resistance-associated positions were considered in the resistance analyses for GT1a and GT1b infected subjects (/ indicates references that differ according to subtype):

- NS3: V36, F43, T54, V55, Y56, V107, S122, I/V132, R155, A156, V158, D168, I/V170
- NS5A: K/Q24, M/L28, P29, Q/R30, L31, P32, H/P58, A92, Y93

Table 68 (FDA analysis) summarizes the proportion of HCV GT1 infected subjects with known NS3/4A or NS5A RASs detected at baseline, based on 15% and 2% sensitivity cutoffs, and according to prior class experience. Not surprisingly, prior NS5A inhibitor experience was more likely to be associated with the presence of baseline NS5A RASs, compared to prior NS3/4A PI experience and baseline NS3 RASs, likely reflecting the more prolonged persistence of NS5A RASs, and possibly also more recent DAA treatment. Based on the ≥15% NGS cutoff, 68/84 (81%) NS5A inhibitor-experienced subjects had an NS5A RAS detected, while 36/94 (38%) NS3/4A PI-experienced subjects had an NS3 RAS detected. Using the more sensitive 2% NGS cutoff increased the proportion of subjects with baseline RASs by <10% for both of these populations (75/84 and 42/94, respectively).

Table 68. Proportion of HCV GT1 infected subjects in MAGELLAN-1 with detectable known RASs at baseline (all arms, non-VF-censored dataset).

15% NGS Sensitivity Cutoff				
Prior DAA Class Experience	**No NS3/4A or NS5A RAS**	**NS3 RAS Only**	**NS5A RAS Only**	**NS3/4A and NS5A RAS**
NS3/4A PI-Exp, NS5A I-Naïve	59% (29/49)	22% (11/49)	8% (4/49)	10% (5/49)
NS5A I-Exp, NS/4A PI-Naïve	15% (6/39)	3% (1/39)	62% (24/39)	21% (8/39)
NS3/4A PI- and NS5A I-Exp	13% (6/45)	7% (3/45)	42% (19/45)	38% (17/45)
2% NGS Sensitivity Cutoff				
Prior DAA Class Experience	**No NS3/4A or NS5A RAS**	**NS3 RAS Only**	**NS5A RAS Only**	**NS3/4A and NS5A RAS**
NS3/4A PI-Exp, NS5A I-Naïve	53% (26/49)	29% (14/49)	4% (2/49)	14% (7/49)
NS5A I-Exp, NS/4A PI-Naïve	10% (4/39)	0% (0/39)	62% (24/39)	28% (11/39)
NS3/4A PI- and NS5A I-Exp	7% (3/45)	4% (2/45)	47% (21/45)	42% (19/45)

Figure 9 (FDA analysis) summarizes the proportion of subjects with HCV amino acid substitutions detected at specific resistance-associated positions according to subjects' prior DAA class history. Here a 2% sensitivity cutoff was considered, not necessarily for clinical utility, but for optimal sensitivity in detecting minority variants to understand the baseline resistance characteristics of the study population.

The imbalance of specific DAA exposure across the different DAA class-exposed subgroups may impact the baseline RASs present, particularly for NS3/4A PIs. Among HCV GT1a infected subjects, the patterns of detected NS3/4A PI RASs varied across these subgroups. Most notably, RASs at NS3 position Y56 were detected in subjects with prior NS3/4A PI and NS5A inhibitor experience, but not in subjects with NS3/4A PI experience only; this likely reflects resistance selection by PTV/r as all 5 subjects with Y56 substitutions previously failed PTV/r-containing treatment. The NS5A RAS patterns were not strikingly different between NS5A inhibitor-experienced subjects, with versus without NS3/4A PI experience, indicating that the NS5A inhibitors used previously in these subgroups had similar resistance patterns. Baseline NS5A RASs were most commonly detected at position Q30 for GT1a infected subjects.

A small proportion of GT1a NS5A inhibitor-naïve subjects had polymorphisms at NS5A resistance-associated positions. However, the most common polymorphism at these positions was H58P, which unlike other changes at this position is generally not associated with failure or treatment-emergence, while several subjects with prior NS5A inhibitor experience had other changes at this position that have been associated with treatment failure (e.g., H58D).

For HCV GT1b infected subjects, there were some differences in the RAS detection patterns according to prior DAA treatment history, but the small numbers of subjects for analysis makes it difficult to draw conclusions.

Of note, no HCV GT1a or GT1b infected subjects had substitutions detected at NS3 position A156, which is a key GLE resistance-associated position based on cell culture resistance characteristics and treatment-emergent resistance patters. Although substitutions at this position have been shown to emerge in some

patients treated with NS3/4A protease inhibitors, they tend not to be persistently detected following treatment (e.g., Howe et al., 2015).

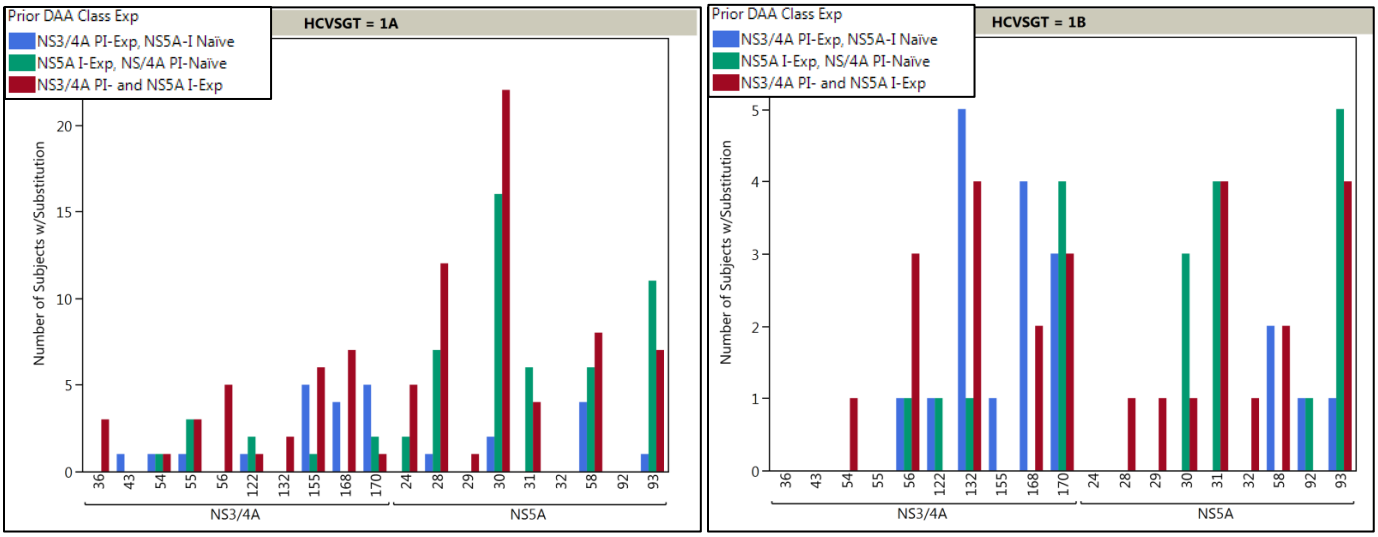


Figure 9. Number of HCV GT1 infected subjects with baseline HCV substitutions/polymorphisms detected at known resistance-associated positions (2% NGS cutoff, non-VF-censored dataset). Positions where substitutions/polymorphisms were not detected are not included in the figure (NS3 V107, A156, V158).

These baseline resistance findings, along with variable disease status and prior treatment histories, indicate that the MAGELLAN-1 study population was heterogeneous. This heterogeneity in the study population, coupled with the relatively small size of the trial, made it challenging to conclusively pinpoint specific host or viral factors associated with treatment failure for the NS5A inhibitor-experienced population.

HCV GT1 Efficacy According to Baseline RAS Patterns

As shown in Table 69 (FDA analysis), no subjects without detected baseline NS5A RASs (based on either NGS detection cutoff) experienced virologic failure. Not surprisingly, most subjects without NS5A RASs were in the NS5A inhibitor-naïve group, among whom none experienced virologic failure in this trial. Overall among subjects who received GLE/PIB for 12W (Arms A,C,D) who were either (a) NS5A inhibitor-naïve, or (b) NS5A inhibitor-experienced without any NS5A RASs (15% cutoff), the SVR12 rate was 32/32 (100%, n=27 GT1a, 5 GT1b).

Table 69. SVR12 (non-VF-censored) rates for HCV GT1 subjects according to the detection of baseline substitutions or polymorphisms at known resistance-associated positions. ND, no data.

HCV Subtype	Regimen Group	Prior DAA Class Exp	No NS3/4A or NS5A RAS	NS3/4A RAS Only	NS5A RAS Only	NS3/4A AND NS5A RAS
15% NGS Cutoff						
1A	GLE/PIB 12W (A,C,D)	NS3/4A PI-Exp, NS5A-I Naïve	100% (15/15)	100% (3/3)	100% (2/2)	1/1
		NS5A I-Exp, NS/4A PI-Naïve	1/1	1/1	91% (10/11)	50% (1/2)
		NS3/4A PI- and NS5A I-Exp	100% (3/3)	1/1	89% (8/9)	67% (4/6)
	GLE/PIB 16W (E)	NS3/4A PI-Exp, NS5A-I Naïve	100% (5/5)	1/1	100% (2/2)	ND
		NS5A I-Exp, NS/4A PI-Naïve	100% (4/4)	ND	83% (5/6)	100% (3/3)
		NS3/4A PI- and NS5A I-Exp	100% (2/2)	1/1	100% (4/4)	25% (1/4)
	GLE/PIB+RBV 12W (B)	NS3/4A PI-Exp, NS5A-I Naïve	100% (7/7)	100% (2/2)	ND	ND
		NS5A I-Exp, NS/4A PI-Naïve	1/1	ND	100% (3/3)	ND
		NS3/4A PI- and NS5A I-Exp	1/1	ND	80% (4/5)	1/1
1B	GLE/PIB 12W (A,C,D)	NS3/4A PI-Exp, NS5A-I Naïve	1/1	100% (3/3)	ND	1/1
		NS5A I-Exp, NS/4A PI-Naïve	ND	ND	100% (3/3)	100% (2/2)
		NS3/4A PI- and NS5A I-Exp	ND	ND	ND	75% (3/4)
	GLE/PIB 16W (E)	NS3/4A PI-Exp, NS5A-I Naïve	1/1	100% (2/2)	ND	1/1
		NS5A I-Exp, NS/4A PI-Naïve	ND	ND	1/1	1/1
		NS3/4A PI- and NS5A I-Exp	ND	1/1	1/1	100% (2/2)
	GLE/PIB+RBV 12W (B)	NS3/4A PI-Exp, NS5A-I Naïve	ND	ND	ND	100% (2/2)
2% NGS Cutoff						
1A	GLE/PIB 12W (A,C,D)	NS3/4A PI-Exp, NS5A-I Naïve	100% (12/12)	100% (6/6)	1/1	100% (2/2)
		NS5A I-Exp, NS/4A PI-Naïve	1/1	ND	91% (10/11)	67% (2/3)
		NS3/4A PI- and NS5A I-Exp	100% (2/2)	1/1	90% (9/10)	67% (4/6)
	GLE/PIB 16W (E)	NS3/4A PI-Exp, NS5A-I Naïve	100% (5/5)	1/1	1/1	1/1
		NS5A I-Exp, NS/4A PI-Naïve	100% (2/2)	ND	86% (6/7)	100% (4/4)
		NS3/4A PI- and NS5A I-Exp	1/1	ND	100% (5/5)	40% (2/5)
	GLE/PIB+RBV 12W (B)	NS3/4A PI-Exp, NS5A-I Naïve	100% (7/7)	100% (2/2)	ND	ND
		NS5A I-Exp, NS/4A PI-Naïve	1/1	ND	100% (3/3)	ND
		NS3/4A PI- and NS5A I-Exp	ND	ND	80% (4/5)	100% (2/2)
1B	GLE/PIB 12W (A,C,D)	NS3/4A PI-Exp, NS5A-I Naïve	1/1	100% (3/3)	ND	1/1
		NS5A I-Exp, NS/4A PI-Naïve	ND	ND	100% (3/3)	100% (2/2)
		NS3/4A PI- and NS5A I-Exp	ND	ND	ND	75% (3/4)
	GLE/PIB 16W (E)	NS3/4A PI-Exp, NS5A-I Naïve	1/1	100% (2/2)	ND	1/1
		NS5A I-Exp, NS/4A PI-Naïve	ND	ND	ND	100% (2/2)
		NS3/4A PI- and NS5A I-Exp	ND	1/1	1/1	100% (2/2)
	GLE/PIB+RBV 12W (B)	NS3/4A PI-Exp, NS5A-I Naïve	ND	ND	ND	100% (2/2)

The NS3 Q80K substitution is common as a baseline polymorphism in GT1a but is not a common treatment-emergent substitution, and as noted in Section 5.2 the presence of the GT1a Q80K polymorphism did not impact GLE/PIB treatment efficacy for NS3/4A PI- and NS5A inhibitor-naïve subjects. Similarly, in MAGELLAN-1 there was no signal that Q80K reduced treatment efficacy in NS3/4A PI- and/or NS5A inhibitor-experienced subjects (Table 70; FDA analysis). Therefore, further resistance analyses did not consider NS3 Q80K.

Table 70. SVR12 (non-VF-censored) rates in MAGELLAN-1 among GT1a infected subjects with or without the NS3 Q80K polymorphism (15% NGS cutoff).

	SVR12 with Q80K	SVR12 without Q80K
All GT1a Subjects, All Arms	93% (42/45)	89% (55/62)
GT1a, NS5A Inhibitor-Experienced, GLE/PIB 12W (Arms A/C/D)	100% (12/12)	77% (17/22)

SVR12 rates for NS5A inhibitor-experienced subjects (with or without NS3/4A PI experience) according to the detection of specific individual RASs are summarized in Table 71 (FDA analysis). More detailed breakdowns of SVR12 rates according to specific RASs detected at 15% and 2% NGS cutoffs are provided in Appendix F and Appendix G, respectively. The presence of substitutions or polymorphisms at several resistance-associated positions was associated with treatment failure. Among HCV GT1a infected subjects, the most common baseline RASs, including NS3 D168(any) and NS5A Q30(any), also captured the most cases of virologic failure. The single GT1b virologic failure subject had a baseline NS5A P32-Deletion, which confers a dramatic 1,036-fold reduction in PIB activity.

As in the analyses shown above, the use of the more sensitive NGS 2% sensitivity cutoff did not have a substantial impact on these baseline resistance analysis results. Subsequent baseline resistance analyses primarily considered NGS data reported at the 15% sensitivity cutoff, which is a more practical sensitivity cutoff for clinical use for the purposes of screening patients to guide treatment decision making.

Table 71. SVR12 rates (non-VF-censored, 15% NGS cutoff) for NS5A inhibitor-experienced subjects with baseline RASs.

HCV Subtype	Target	Baseline Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
1a	NS3	**V36L/M**	1/2	1/1	ND	**67% (2/3)**
		T54S	1/1	1/1	ND	**100% (2/2)**
		V55A/I	2/4	1/1	ND	**60% (3/5)**
		Y56H	1/1	0/2	ND	**33% (1/3)**
		S122G	1/1	1/1	ND	**100% (2/2)**
		I132L	1/1	ND	ND	**1/1**
		R155K/T	3/4	1/1	ND	**80% (4/5)**
		D168A/E/N/T/V	1/2	1/4	1/1	**43% (3/7)**
		I170V	ND	3/3	ND	**100% (3/3)**
	NS5A	**K24Q/R**	3/3	0/1	ND	**75% (3/4)**
		M28A/T/V	7/8	2/3	1/1	**83% (10/12)**
		Q30E/G/H/K/L/R	12/17	6/9	6/6	**75% (24/32)**
		L31M/V	4/4	2/4	1/2	**70% (7/10)**
		H58C/D/P/Q/Y	5/7	3/3	0/1	**73% (8/11)**
		Y93H/N	5/5	6/8	4/4	**88% (15/17)**
1b	NS3	**T54S**	ND	1/1	ND	**1/1**
		Y56F	0/1	3/3	ND	**75% (3/4)**
		S122T	ND	1/1	ND	**1/1**
		V132I/L	2/2	3/3	ND	**100% (5/5)**
		V170I	3/3	3/3	ND	**100% (6/6)**
	NS5A	**L28M**	0/1	ND	ND	**0/1**
		R30K/Q	1/1	1/1	ND	**100% (2/2)**
		L31I/M/V	6/6	2/2	ND	**100% (8/8)**
		P32-Deletion	0/1	ND	ND	**0/1**
		P58S	1/1	1/1	ND	**100% (2/2)**
		A92E	1/1	ND	ND	**1/1**
		Y93H/S	5/5	4/4	ND	**100% (9/9)**

Table 72 (FDA analysis) summarizes SVR12 rates according to the detection of baseline NS5A RASs considering the "Primary" and "Extended" lists of NS5A amino acid positions described in Section 5.2 for analyses of NS3/4A PI- and NS5A inhibitor-naïve subjects (i.e., for analyses of NS5A polymorphisms). These lists do not appear to be particularly useful for NS5A inhibitor-experienced subjects given the high frequency of detection of baseline NS5A RASs in this population. Not surprisingly, all subjects without any detected baseline RASs from either list achieved SVR12.

Table 72. SVR12 rates (non-VF-censored) for HCV GT1a and GT1b infected subjects according to the detection of baseline NS5A RASs, considering "Primary" and "Extended" lists of NS5A amino acid positions (pooling all arms, 15% NGS cutoff).

HCV Subtype	Prior DAA Class Exp	NS5A Any (24,28,30,31,58,92,93) ("Extended List")		NS5A Any (28,30,31,93) ("Primary List")	
		With RAS	Without RAS	With RAS	Without RAS
1a	NS3/4A PI-Exp, NS5A-I Naïve	100% (5/5)	100% (33/33)	100% (2/2)	100% (36/36)
	NS5A I-Exp, NS/4A PI-Naïve	88% (22/25)	100% (7/7)	88% (22/25)	100% (7/7)
	NS3/4A PI- and NS5A I-Exp	76% (22/29)	100% (8/8)	73% (19/26)	100% (11/11)
1b	NS3/4A PI-Exp, NS5A-I Naïve	100% (4/4)	100% (7/7)	1/1	100% (10/10)
	NS5A I-Exp, NS/4A PI-Naïve	100% (7/7)	ND	100% (7/7)	ND
	NS3/4A PI- and NS5A I-Exp	86% (6/7)	1/1	86% (6/7)	1/1

Since most NS5A inhibitor-experienced subjects had multiple baseline RASs across both drug targets, confounding the results shown in the above analyses, a comprehensive 'map' of substitutions at resistance-associated positions was generated for each subject for more optimal identification of baseline resistance patterns associated with virologic failure. This analysis again focused on NS5A inhibitor-experienced subjects, with or without prior NS3/4A PI experience, who received GLE/PIB for 12 or 16 weeks. Results at the 15% NGS sensitivity cutoff are shown in Table 73 (FDA analysis).

Consistent with the data shown above, the map in Table 73 indicates that NS3 D168 and NS5A Q30 baseline RASs were associated with virologic failure for HCV GT1a infected subjects. All but one of the HCV GT1a infected subjects who experienced virologic failure with GLE/PIB had an NS5A Q30 RAS. Either confounding or contributing to these results, the detection of ≥2 NS5A RASs was also associated with virologic failure. Again, similar results were observed using the 2% cutoff (Appendix H), although a few additional baseline RASs were detected, both in virologic failure subjects and SVR12 subjects. The single GT1b virologic failure subject had baseline NS5A RASs (L28M + P32-Deletion) that were not detected among GT1b infected subjects who achieved SVR12.

Table 73. Individual subject-level map of NS3 and NS5A positions with detected baseline RASs for NS5A-experienced subjects who received GLE/PIB for 12 or 16 weeks (15% NGS cutoff, non-VF censored). Red indicates a substitution (relative to GT1a or GT1b reference) was detected at that position.

						NS3									NS5A										
GT	SVR	Prior DAA Class Exp	Regimen	CIRR	USUBJID (O=on-tx VF, R=Relapse)	36	54	55	56	122	132	155	168	170	24	28	30	31	32	58	92	93	# NS3 BL RAS	# NS5A BL RAS
1a	N	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-53286-2924 (R)	0	0	A	0	0	0	0	0	0	0	0	R	0	0	D	0	0	1	2
				Y	M15410-38627-1321 (O)	0	0	0	0	0	0	0	0	0	0	0	E/K	0	0	0	0	0	0	1
			GP 16W	Y	M15410-42371-2021 (O)	0	0	0	0	0	0	0	0	0	0	0	R	M	0	0	0	0	0	2
		NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-40621-3023 (R)	0	0	0	0	0	0	0	0	0	0	0	G	0	0	0	0	0	0	1
				N	M15410-41723-2605 (O)	0	0	0	0	0	0	0	A	0	0	0	R	0	0	C	0	0	1	2
				N	M15410-41723-2624 (R)	M	0	I	0	0	0	K	0	0	0	V	R	0	0	0	0	0	3	2
			GP 16W	N	M15410-41723-2622 (O)	0	0	0	H	0	0	0	A	0	0	T/V	R	M	0	0	0	0	2	3
				Y	M15410-12641-1620 (O)	0	0	0	H	0	0	0	E	0	Q	0	0	0	0	0	0	H	2	2
				Y	M15410-40621-3025 (O)	0	0	0	0	0	0	0	A	0	0	0	H	0	0	0	0	H	1	2
	Y	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-22887-2120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	1
				N	M15410-22887-2122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Y	0	N	0	2
				N	M15410-37834-2401	0	0	0	0	0	0	0	0	0	0	0	0	M	0	0	0	0	0	1
				N	M15410-38627-1322	0	0	0	0	0	0	0	0	0	0	0	0	M	0	0	0	0	0	1
				N	M15410-38853-2223	0	0	0	0	0	0	0	0	0	R	T	H	0	0	P	0	0	0	4
				N	M15410-40621-3027	0	0	A	0	0	0	0	0	0	0	A	R	0	0	0	0	0	1	2
				N	M15410-41723-2602	0	0	0	0	0	0	K	0	0	0	0	0	0	0	0	0	0	1	0
				N	M15410-42362-1520	0	0	0	0	0	0	0	0	0	0	T	H	0	0	0	0	0	0	2
				N	M15410-50807-2801	0	0	0	0	0	0	0	0	0	0	0	H	0	0	0	0	H	0	2
				N	M15410-53286-2920	0	0	0	0	0	0	0	0	0	0	T	H	0	0	0	0	0	0	2
				N	M15410-41723-2620	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Y	M15410-37759-4526	0	0	0	0	0	0	0	0	0	0	0	E	0	0	0	0	0	0	1
				Y	M15410-53286-2922	0	0	0	0	0	0	0	0	0	0	0	0	M	0	P	0	0	0	2
			GP 16W	N	M15410-37759-4527	0	0	0	0	0	0	0	0	0	0	0	R	M	0	0	0	0	0	2
				N	M15410-38627-1320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	1
				N	M15410-38853-2220	0	0	0	0	0	0	0	0	0	0	0	H	0	0	0	0	H	0	2
				N	M15410-38853-2222	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	1
				N	M15410-40621-3024	0	0	0	0	0	0	0	0	0	0	A	R	0	0	0	0	0	0	2
				N	M15410-41723-2621	0	0	0	0	G	0	0	0	V	0	0	0	0	0	0	0	N	2	1
				N	M15410-40621-3031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-40622-5020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-53286-2921	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-76269-4820	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Y	M15410-42730-1025	0	0	0	0	0	0	0	0	V	0	0	0	M	0	P	0	0	1	2
				Y	M15410-76269-4821	0	S	I	0	0	0	0	0	0	0	0	0	0	0	0	0	N	2	1
		NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-18161-1208	0	0	0	0	0	0	0	0	0	0	V	H	0	0	0	0	0	0	2
				N	M15410-12641-1619	0	0	0	0	G	0	0	0	0	0	0	0	0	0	0	0	0	1	0
				N	M15410-12641-1622	0	0	0	0	0	0	0	0	0	0	0	E	0	0	0	0	0	0	1
				N	M15410-18161-1221	0	0	0	0	0	0	0	0	0	0	0	H	0	0	0	0	H/N	0	2
				N	M15410-35827-1901	0	0	0	0	0	0	0	0	0	R	0	0	0	0	0	0	0	0	1
				N	M15410-37739-1805	0	0	0	0	0	0	0	0	0	0	0	E	0	0	0	0	0	0	1
				N	M15410-37739-1806	0	0	0	0	0	0	0	0	0	0	0	0	M	0	P	0	0	0	2
				N	M15410-37759-4521	0	0	0	0	0	0	0	0	0	0	T	0	0	0	0	0	0	0	1
				N	M15410-38627-1305	M	S	0	0	0	0	0	0	0	R	0	R	0	0	0	0	0	2	2
				N	M15410-40621-3030	0	0	A	0	0	0	0	0	0	0	0	0	0	0	Y	0	0	1	1
				N	M15410-51321-4422	0	0	0	H	0	L	T	N	0	0	T	0	0	0	0	0	0	4	1
				N	M15410-51382-1402	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	1
				N	M15410-40621-3022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-40622-5022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-44319-3725	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				Y	M15410-44318-3623	0	0	0	0	0	0	K	0	0	0	0	E	0	0	0	0	0	1	1
			GP 16W	N	M15410-12641-1621	0	0	0	0	0	0	0	0	0	0	0	H	0	0	0	0	0	0	1
				N	M15410-37739-1820	0	0	0	0	0	0	0	0	0	0	T	0	0	0	Q	0	0	0	2
				N	M15410-40621-3021	0	0	0	0	0	0	0	0	0	0	0	E	0	0	0	0	0	0	1
				N	M15410-44319-3724	0	0	0	0	0	0	K	0	V	0	0	0	0	0	0	0	0	2	0
				N	M15410-44372-4020	L	0	0	0	0	0	0	V	0	0	0	H	0	0	0	0	H	2	2
				N	M15410-92059-3420	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	0	0	0	1
				N	M15410-41723-2623	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				N	M15410-42730-1024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1b	N	NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-44372-4021 (R)	0	0	0	F	0	0	0	0	0	0	M	0	0	del	0	0	0	1	2
	Y	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-38645-2501	0	0	0	0	0	0	0	0	0	0	0	0	I	0	0	0	H	0	2
				Y	M15410-22887-2125	0	0	0	0	0	0	0	0	I	0	0	K	M	0	0	E	0	1	3
				Y	M15410-38853-2225	0	0	0	0	0	0	0	0	I	0	0	0	/M	0	0	0	H	1	2
				Y	M15410-40621-3020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H	0	1
				Y	M15410-40724-3921	0	0	0	0	0	0	0	0	0	0	0	0	M	0	0	0	H	0	2
			GP 16W	N	M15410-38853-2226	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H	0	1
				N	M15410-47163-3823	0	0	0	F	T	I	0	0	I	0	0	Q	0	0	0	0	0	4	1
		NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-18161-1209	0	0	0	0	0	I	0	0	0	0	0	0	M	0	0	0	0	1	1
				N	M15410-51382-1401	0	0	0	0	0	I	0	0	0	0	0	0	M	0	0	0	0	1	1
				N	M15410-40724-3923	0	0	0	0	0	0	0	0	I	0	0	0	0	0	S	0	S	1	2
			GP 16W	N	M15410-40621-3032	0	0	0	F	0	0	0	0	I	0	0	0	0	0	0	0	0	2	0
				N	M15410-44319-3720	0	S	0	0	0	I	0	0	I	0	0	0	M	0	0	0	H	3	2
				N	M15410-44319-3722	0	0	0	0	0	0	0	0	0	0	0	0	V	0	0	0	H	0	2
				Y	M15410-40724-3922	0	0	0	F	0	I	0	0	0	0	0	0	0	0	S	0	H	2	2

Among NS3/4A PI-experienced, NS5A inhibitor-naïve subjects the presence of baseline NS3 RASs apparently did not affect treatment outcome, as all subjects in this subgroup achieved SVR12. To better understand the specific NS3 RASs represented in this subgroup, which may inform the potential contribution of NS3 RASs toward treatment failure in the NS3/4A PI- and NS5A inhibitor-experienced subgroup, an additional map of NS3 RASs and NS5A polymorphisms was created for NS3/4A PI-experienced, NS5A inhibitor-naïve subjects.

As shown in Table 74 (FDA analysis), only one NS3/4A PI-experienced, NS5A inhibitor-naïve, HCV GT1a infected subject had an NS3 D168 RAS (D168V) detected at the ≥15% level, with no NS5A resistance-associated polymorphisms detected. Not surprisingly, only a subset of these NS5A inhibitor-naïve subjects had NS5A polymorphisms of interest, most of which occurred at position H58 and included specific polymorphisms (e.g., H58P) that were not associated with virologic failure in NS5A inhibitor-experienced subjects.

Interestingly, one GT1a NS3/4A PI-experienced/NS5A inhibitor-naïve subject (Subject 4620) had baseline NS5A Q30H + Y93H RASs (i.e., polymorphisms) detected but no NS3 baseline RASs, and achieved SVR12. Among NS5A inhibitor-experienced subjects, 5 subjects had this same combination of baseline RASs. Three of these 5 subjects similarly had no NS3 baseline RASs and achieved SVR12. The 2 other NS5A inhibitor-experienced subjects with baseline NS5A Q30H + Y93H had an NS3 D168 RAS (D168A or D168V), and one of these subjects experienced virologic failure. These results are consistent with combinations of NS3 and NS5A RASs contributing to virologic failure among subjects with prior exposure to both DAA classes.

Table 74. Individual subject-level map of NS3 and NS5A positions with detected baseline RASs for NS3/4A PI-experienced, NS5A inhibitor-naïve subjects (15% NGS cutoff, non-VF censored). Red indicates a substitution (relative to GT1a or GT1b reference) was detected. The table does not include 29 subjects without any detected RASs in NS3 or NS5A. All NS3/4A PI-experienced, NS5A inhibitor-naïve subjects achieved SVR12.

					NS3								NS5A					
GT	Regimen Group	CIRR	USUBJID	Prior PI Use	54	55	56	122	132	155	168	170	30	58	92	93	# NS3 BL RAS	# NS5A BL RAS
1a	GLE/PIB 12W (A,C,D)	N	M15410-14446-4123	BOC	0	0	0	0	0	K	0	0	0	P	0	0	1	1
		N	M15410-40621-3026	TVR	0	0	0	0	0	0	0	0	0	P	0	0	0	1
		N	M15410-40621-3029	TVR	0	A	0	0	0	0	0	0	0	0	0	0	1	0
		N	M15410-92059-3421	SMV	0	0	0	0	0	0	V	V	0	0	0	0	2	0
		Y	M15410-40621-3033	BOC	S	0	0	0	0	K	0	0	0	0	0	0	2	0
		Y	M15410-40621-3034	TVR	0	0	0	0	0	0	0	0	H	P	0	0	0	2
	GLE/PIB 16W (E)	N	M15410-06381-4620	BOC	0	0	0	0	0	0	0	0	H	0	0	H	0	2
		N	M15410-38853-2224	BOC	0	0	0	0	0	0	0	0	0	P	0	0	0	1
		N	M15410-40621-3028	TVR	0	0	0	0	0	K	0	V	0	0	0	0	2	0
	GLE/PIB+RBV 12W (B)	N	M15410-50807-2802	BOC	0	0	0	0	0	0	0	V	0	0	0	0	1	0
		N	M15410-50807-2805	BOC	0	0	0	G	0	0	0	0	0	0	0	0	1	0
1b	GLE/PIB 12W (A,C,D)	N	M15410-37739-1803	PTV/r	0	0	0	0	0	0	E	0	0	0	T	0	1	1
		N	M15410-38627-1302	TVR	0	0	0	0	I	0	0	0	0	0	0	0	1	0
		N	M15410-38934-2701	TVR	0	0	0	0	I	0	0	0	0	0	0	0	1	0
		N	M15410-47163-3824	TVR	0	0	0	0	I	0	0	0	0	0	0	0	1	0
	GLE/PIB 16W (E)	N	M15410-40621-3036	SMV	0	0	0	0	0	0	E/V	0	0	0	0	0	1	0
		Y	M15410-47163-3822	SMV	0	0	F	0	0	0	V	I	0	0	0	0	3	0
		Y	M15410-51321-4421	SMV	0	0	0	G	0	0	0	0	0	T	0	0	1	1
	GLE/PIB+RBV 12W (B)	N	M15410-22887-2102	BOC	0	0	0	0	0	0	0	I	0	0	0	H	1	1
		N	M15410-48698-1703	SMV	0	0	0	0	I	0	V	0	0	S	0	0	2	1

Additional analyses were conducted to assess if baseline phenotypic susceptibilities to GLE and PIB are predictive of virologic failure. Direct phenotypic analyses of clinical isolates were not conducted, and therefore baseline phenotype was predicted based on site-directed mutant HCV replicon phenotype data for individual amino acid substitutions (Appendix A and Appendix B). Site-directed mutant replicon phenotype data were not available for certain individual or combinations of RASs, and in such cases phenotype had to be inferred based on other available data.

Table 75 (FDA analysis) summarizes predicted GLE and PIB phenotypic susceptibilities of baseline viruses from the 11 subjects who experienced virologic failure in MAGELLAN-1. The predicted GLE and PIB phenotypic susceptibilities varied tremendously across the different subjects. While some subjects had baseline viruses with large reductions in GLE or PIB phenotypic susceptibility (e.g., Subject 1620 for GLE and Subject 4021 for PIB), many subjects did not, especially considering the sub-picomolar activity of PIB against wild-type HCV replicons. Therefore, the lack of baseline phenotypic resistance to GLE or PIB does not necessarily predict GLE/PIB will be effective in this population. As discussed below in the treatment-emergent resistance summary, it appears that many of the baseline NS3 or NS5A RASs contributed to virologic failure, not by conferring phenotypic resistance by themselves, but by reducing the resistance barrier to make it easier for the virus to acquire combinations of amino acid substitutions that collectivley confer phenotypic resistance.

Table 75. Predicted phenotypic susceptibility of baseline viruses according to RAS patterns detected (NGS 15% cutoff) for subjects who experienced virologic failure in MAGELLAN-1. Parentheses in the fold-change columns indicate that fold-change data were imputed based on the amino acid substitutions shown due to lack of available phenotype data for specific RASs detected in the clinical sample.

USUBJID (O=on-tx VF, R=Relapse)	NS3 BL RAS	GLE Fold-Change	NS5A BL RAS	PIB Fold-Change
M15410-38627-1321 (O)	none	1	Q30E/K	2, 1 (Q30E,Q30K)
M15410-42371-2021 (O)	none	1	Q30R+L31M	3
M15410-40621-3023 (R)	none	1	Q30G	1
M15410-41723-2601 (R)	none	1	L31M+H58D	23
M15410-41723-2605 (O)	D168A	4	Q30R+H58C	126 (Q30R+H58**D**)
M15410-41723-2624 (R)	V36M+V55I+R155K	<1 (V36M+R155K)	M28V+Q30R	1
M15410-41723-2622 (O)	Y56H+D168A	39	M28T/V+Q30R+L31M	5 (M28T+Q30R+L31M)
M15410-12641-1620 (O)	Y56H+D168E	47	K24Q+Y93H	7 (Y93H)
M15410-40621-3025 (O)	D168A	4	Q30H+Y93H	17
M15410-53286-2924 (R)	V55A	0.2 (V55I)	Q30R+H58D	126
M15410-44372-4021 (R) (GT1b)	Y56F	No Data for Y56-any in GT1b	L28M+P32del	6326

HCV GT1 Treatment-Emergent Resistance

All 11 HCV GT1 infected subjects who experienced virologic failure (10 GT1a, 1, GT1b) had baseline and post-baseline data available to conduct a treatment-emergent resistance analysis; however, only NS3 sequence analysis data (i.e., no NS5A data) were available from a post-baseline visit from Subject 2622 (GT1a). Table 76 (FDA analysis) provides a subject-level tabulation of baseline and post-baseline RASs detected at any level (≥2%), and Table 77 (FDA analysis) provides a summary of treatment-emergent substitutions. Note that the treatment-emergent substitutions listed also include those that were detected at baseline (≥2%) but were enriched in frequency by ≥20% post-baseline (per sponsor's definition); nevertheless, the vast majority of treatment-emergent substitutions were not detected at baseline.

Despite the presence of baseline NS5A RASs in all virologic failure subjects, many of whom also had baseline NS3 RASs, treatment failure was associated with the emergence of additional or evolving RASs in NS3 and NS5A. Among HCV GT1a virologic failure subjects, 9/10 (90%) subjects had treatment-emergent or evolving RASs in NS3 or NS5A, and 7/9 (78%) subjects with available data had treatment-emergent or evolving RASs in both targets.

Table 76. Treatment-emergent resistance analysis for subjects who experienced virologic failure in MAGELLAN-1. Emergent substitutions include those that were not detected (<2%) at baseline. Enriched substitutions include those that were detected at baseline (≥2%) but enriched by >20% prevalence at the post-baseline analysis timepoint.

Subject	GT	Cirr?	DAAs Exper.	Arm	Regimen	Visit	Target	RAS	% Freq	Emrg?	Enrch?	Tx Emerg Summary
M15410-12641-1620	1a	Y	NS3/4A PI- and NS5A I-Exp	E	G/P (300/120) 16W	BL	NS3/4A	Y56H	97.3			NS3 and NS5A RAS(s) Emerged
								D168E	94.8			
							NS5A	K24Q	99.8			
								Y93H	98.7			
						Post-BL	NS3/4A	V36M	97.8	Y		
								Y56H	99.4			
								D168E	99.4			
							NS5A	K24Q	81.1			
								K24R	18.7	Y		
								Q30K	95.7	Y		
								H58D	4.3	Y		
								Y93H	99			
M15410-38627-1321	1a	Y	NS5A I-Exp, NS/4A PI-Naïve	D	G/P (300/120) 12W	BL	NS5A	K24E	2			NS3 and NS5A RAS(s) Emerged
								M28V	3.5			
								Q30E	66			
								Q30K	29.3			
								Q30R	3.6			
						Post-BL	NS3/4A	A156V	99.8	Y		
							NS5A	P29Q	2.1	Y		
								Q30K	99.7		Y	
								Y93H	97.6	Y		
								Y93N	2.1	Y		
M15410-40621-3023	1a	N	NS3/4A PI- and NS5A I-Exp	D	G/P (300/120) 12W	BL	NS5A	Q30E	4.9			NS5A RAS(s) Emerged
								Q30G	93.5			
						Post-BL	NS5A	P29R	99.5	Y		
								Q30G	99			
M15410-40621-3025	1a	Y	NS3/4A PI- and NS5A I-Exp	E	G/P (300/120) 16W	BL	NS3/4A	Y56H	12.6			NS3 and NS5A RAS(s) Emerged
								R155T	2.1			
								D168A	25.5			
								D168T	3.1			
							NS5A	Q30H	99.4			
								Y93H	98.7			
						Post-BL	NS3/4A	R155T	99.9		Y	
								A156V	99.9	Y		
								D168A	99.9		Y	
							NS5A	M28A	29	Y		
								Q30H	99.7			
								H58D	70.8	Y		
								Y93H	99.6			
M15410-41723-2601	1a	N	NS3/4A PI- and NS5A I-Exp	B	G/P (300/120) + RBV 12W	BL	NS5A	L31M	99.6			NS3 and NS5A RAS(s) Emerged
								H58D	26.3			
						Post-BL	NS3/4A	A156V	90.8	Y		
							NS5A	Q30R	99.5	Y		
								L31M	99.8			
								H58D	99.2		Y	
M15410-41723-2605	1a	N	NS3/4A PI- and NS5A I-	C	G/P (300/120) 12W	BL	NS3/4A	Y56H	4.6			NS3 and NS5A RAS(s)
								D168A	94.1			
								D168T	2.9			

			Exp				NS5A	M28V	3.1			Emerged
								Q30R	98			
								H58C	98.9			
						Post-BL	NS3/4A	V36M	6.5	Y		
								Y56H	99.4		Y	
								D168A	99.8			
							NS5A	M28G	99.4	Y		
								Q30R	98.9			
								H58C	99.7			
M15410-41723-2622	1a	N	NS3/4A PI- and NS5A I-Exp	E	G/P (300/120) 16W	BL	NS3/4A	Y56H	98.9			NS3 RAS(s) Emerged, no data for NS5A
								D168A	98.3			
							NS5A	M28T	41.1			
								M28V	35.1			
								Q30L	2.2			
								Q30R	97.6			
								L31M	99.5			
								H58D	4.7			
						Post-BL	NS3/4A	V36A	4.5	Y		
								V36M	5.1	Y		
								Y56H	99.4			
								A156G	89.2	Y		
								D168A	94.1			
								D168T	5.8	Y		
							NS5A	No Post-BL NS5A Data Available				
M15410-41723-2624	1a	N	NS3/4A PI- and NS5A I-Exp	D	G/P (300/120) 12W	BL	NS3/4A	V36M	99.3			NS3 and NS5A RAS(s) Emerged
								V55I	60.8			
								R155K	41.5			
							NS5A	M28V	98.1			
								Q30R	96.2			
						Post-BL	NS3/4A	V36M	99.9			
								V55I	2.5			
								R155K	98.3		Y	
								A156T	98.2	Y		
							NS5A	M28G	98.8	Y		
								Q30R	98.8			
M15410-42371-2021	1a	Y	NS5A I-Exp, NS/4A PI-Naïve	E	G/P (300/120) 16W	BL	NS5A	Q30R	98.9			NS3 and NS5A RAS(s) Emerged
								L31M	99.7			
						Post-BL	NS3/4A	I132V	5.4	Y		
								A156V	99.9	Y		
							NS5A	Q30R	99.5			
								L31M	99.8			
								H58D	99.2	Y		
M15410-53286-2924	1a	N	NS5A I-Exp, NS/4A PI-Naïve	D	G/P (300/120) 12W	BL	NS3/4A	V55A	99.8			No NS3 or NS5A RAS Emerged
							NS5A	Q30R	97.3			
								H58D	98.2			
						Post-BL	NS3/4A	V55A	99.1			
							NS5A	Q30R	99			
								H58D	98.8			
M15410-44372-4021	1b	N	NS3/4A PI- and NS5A I-Exp	D	G/P (300/120) 12W	BL	NS3/4A	Y56F	98.8			No NS3 or NS5A RAS Emerged (NS5A L28M Enriched)
							NS5A	L28M	28.4			
								P32-	92.1			
						Post-BL	NS3/4A	Y56F	99.8			
							NS5A	L28M	99.8		Y	
								P32-	99.8			

Table 77. Treatment-emergent resistance-associated substitutions among subjects who experienced virologic failure in MAGELLAN-1. Listing includes substitutions those that were not detected (<2%) at baseline and emerged post-baseline, as well as enriched substitutions that were detected at baseline (≥2%) but enriched by ≥20% prevalence post-baseline.

HCV Subtype	Target	Position	Substitution	# (%) subjects with emerged or enriched substitution
1a	NS3/4A	36	V36A	10% (1/10)
			V36M	30% (3/10)
			V36A/M	**30% (3/10)**
		56	**Y56H**	**10% (1/10)**
		132	**I132V**	**10% (1/10)**
		155	R155K	10% (1/10)
			R155T	10% (1/10)
			R155K/T	**20% (2/10)**
		156	A156G	10% (1/10)
			A156T	10% (1/10)
			A156V	40% (4/10)
			A156G/T/V	**60% (6/10)**
		168	D168A	10% (1/10)
			D168T	10% (1/10)
			D168A/T	**20% (2/10)**
	NS5A	24	K24R	11% (1/9)
		28	M28A	11% (1/9)
			M28G	22% (2/9)
			M28A/G	**33% (3/9)**
		29	P29Q	11% (1/9)
			P29R	11% (1/9)
			P29Q/R	**22% (2/9)**
		30	Q30K	22% (2/9)
			Q30R	11% (1/9)
			Q30K/R	**33% (3/9)**
		58	**H58D**	**44% (4/9)**
		93	Y93H	11% (1/9)
			Y93N	11% (1/9)
			Y93H/N	**11% (1/9)**
	Any NS3/4A RAS			**80% (8/10)**
	Any NS5A RAS			**89% (8/9)**
	Any NS3/4A or NS5A RAS			**90% (9/10)**
	NS3/4A AND NS5A RAS			**78% (7/9)**
1b	NS5A	28	L28M	1/1

Phenotypic analyses based on site-directed mutant HCV replicon data indicate that the emergence of additional NS3 and NS5A RASs likely contributed to enhanced GLE and PIB resistance (Table 78; FDA analysis). In many cases viruses at baseline had 1 or more RASs that do not confer significant phenotypic resistance, with the emergence of additional RAS(s) at the time of virologic failure that substantially increase phenotypic resistance. This is most striking for NS5A and PIB activity. Among the 10 subjects with available NS5A data, all 10 (100%) had baseline NS5A RASs, and 8/10 (80%) had treatment-emergent NS5A RASs.

Among these 8 subjects, 7 had phenotype data indicating the additional emergent NS5A RASs resulted in major increases in PIB resistance. The only two subjects without additional emergent NS5A RASs already had baseline RASs that cause a major reduction in PIB susceptibility (126-fold and 6,326 fold), which may explain why additional RASs did not emerge. Similar patterns were observed with NS3 RASs and GLE resistance, although the findings were not quite as clear due to limited GLE phenotype data for the NS3 RASs observed.

Table 78. Predicted GLE and PIB HCV phenotypic susceptibility according to RAS patterns detected (NGS 15% cutoff) at baseline and at the time of virologic failure in MAGELLAN-1. Parentheses indicate that fold-change data were imputed based on the amino acid substitutions shown due to lack of available phenotype data for the specific RASs detected in the clinical sample. Underlined RASs indicate those that were detected ≥15% at the time of failure but not at baseline. FC, fold-change.

Glecaprevir (NS3) Phenotypic Analysis				
USUBJID (O=on-tx VF, R=Relapse)	**Baseline**		**Virologic Failure**	
	NS3 RAS (≥15%)	**GLE FC**	**NS3 RAS (≥15%)**	**GLE FC**
M15410-38627-1321 (O)	none	1	A156V	<1, 1361, 10922 (A156G,A156T,Q89R+A156V)
M15410-42371-2021 (O)	none	1	A156V	<1, 1361, 10922 (A156G,A156T,Q89R+A156V)
M15410-40621-3023 (R)	none	1	none	1
M15410-41723-2601 (R)	none	1	A156V	<1, 1361, 10922 (A156G,A156T,Q89R+A156V)
M15410-41723-2605 (O)	D168A	4	Y56H+D168A	39
M15410-41723-2624 (R)	V36M+V55I+R155K	<1 (V36M+R155K)	V36M+R155K+A156T	1361 (A156T)
M15410-41723-2622 (O)	Y56H+D168A	39	Y56H+A156G+D168A	39, 32 (Y56H+D168A,A156G+D168A)
M15410-12641-1620 (O)	Y56H+D168E	47	V36M+Y56H+D168E	127
M15410-40621-3025 (O)	D168A	4	R155T+A156V+D168A	2, <1, 1361, 28, 32 (R155T,A156G,A156T,R155T+ D168N, A156G+D168A)
M15410-53286-2924 (R)	V55A	<1 (V55I)	V55A	<1 (V55I)
M15410-44372-4021 (R) (GT1b)	Y56F	No Data for Y56x in GT1b	Y56F	No Data for Y56x in GT1b
Pibrentasvir (NS5A) Phenotypic Analysis				
USUBJID (O=on-tx VF, R=Relapse)	**Baseline**		**Virologic Failure**	
	NS5A RAS (≥15%)	**PIB FC**	**NS5A RAS (≥15%)**	**PIB FC**
M15410-38627-1321 (O)	Q30E/K	2, 1 (Q30E,Q30K)	Q30K+Y93H	1786
M15410-42371-2021 (O)	Q30R+L31M	3	Q30R+L31M+H58D	1704
M15410-40621-3023 (R)	Q30G	1	P29R+Q30G	No Relevant Phenotype Data
M15410-41723-2601 (R)	L31M+H58D	23	Q30R+L31M+H58D	1704
M15410-41723-2605 (O)	Q30R+H58C	126 (Q30R+H58D)	M28G+Q30R+H58C	942
M15410-41723-2624 (R)	M28V+Q30R	1	M28G+Q30R	21824
M15410-41723-2622 (O)	M28T/V+Q30R+L31M	5 (M28T+Q30R+L31M)	No Data	No Data
M15410-12641-1620 (O)	K24Q+Y93H	7 (Y93H)	K24Q/R+Q30K+Y93H	1786 (Q30K+Y93H)
M15410-40621-3025 (O)	Q30H+Y93H	17	M28A+Q30H+H58D+Y93H	9357 (Q30H+H58D+Y93H)
M15410-53286-2924 (R)	Q30R+H58D	126	Q30R+H58D	126
M15410-44372-4021 (R) (GT1b)	L28M+P32del	6326	L28M+P32del	6326

Overall, these results indicate that baseline RASs that do not confer GLE or PIB phenotypic resistance by themselves can still contribute to virologic failure by reducing the resistance barrier of the regimen. Nucleotide errors are generated spontaneously during HCV replication. The presence of a predominant viral population with one or more DAA RAS(s) would increase the probability that HCV variants carrying combinations of RASs that confer enhanced phenotypic resistance are generated during replication. These underlying, DAA-resistant viral populations with complex combinations of RASs are then selected by treatment and emerge at the time of virologic failure.

Potential Baseline Screening Algorithms for GT1a, NS5A Inhibitor-Experienced Patients

A group of baseline RAS algorithms was considered to summarize the baseline resistance analyses in a manner that is potentially useful for guiding treatment decisions for GT1a, NS5A inhibitor treatment-experienced subjects (Table 79; FDA analysis).

In this reviewer's opinion, considering the combination of NS3 D168 and NS5A Q30 baseline RASs is best supported for clinical decision making for this population. The NS3 D168 RASs were detected only in subjects who also had prior NS3/4A PI experience, and was associated with a 43% (3/7) SVR12 rate across all arms. Furthermore, all but 2 of the virologic failure subjects across all arms had a BL NS5A Q30 RAS, reflecting a 75% (24/32) SVR12 rate. Considering the proposed GLE/PIB 16-week treatment for NS5A inhibitor-experienced patients, 10/24 (42%) GT1a subjects had an NS3 D168 or NS5A Q30 RAS, of whom 6 (60%) achieved SVR12 with this regimen. All 14 (100%) GT1a, NS5A inhibitor-experienced subjects without an NS3 D168 or NS5A Q30 RAS who received GLE/PIB for 16 weeks achieved SVR12.

As additional support for NS5A Q30 being a key PIB resistance-associated position, the two HCV GT1a infected subjects without a Q30 RAS who experienced virologic failure had a treatment-emergent Q30 RAS at the time of failure. Furthermore, the only two NS3/4A PI- and NS5A inhibitor-naïve, GT1a infected subjects who experienced virologic failure with to-be-marketed dose levels of GLE/PIB across Phase 2b/3 trials also had treatment-emergent/-enriched NS5A Q30R (Table 33). Thus, 100% of GT1a subjects who experienced virologic failure with GLE/PIB administered at to-be-marketed dose levels in clinical trials had either baseline or treatment-emergent NS5A Q30 RAS(s). Two additional GT1a subjects failed treatment with lower GLE or PIB dose levels in the Phase 2 SURVEYOR-1 trial (Table 28), and one of these subjects also had a treatment-emergent Q30 RAS.

Table 79. SVR12 rates according to various baseline RAS detection algorithms for HCV GT1a infected, NS5A inhibitor-experienced subjects (non-VF censored, 15% NGS cutoff).

Subpopulation		GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
Baseline NS3 D168 RAS					
NS5A I-Exp, NS/4A PI-Naïve	No NS3 D168 RAS	87% (13/15)	92% (12/13)	100% (4/4)	91% (29/32)
NS3/4A PI- and NS5A I-Exp	No NS3 D168 RAS	88% (15/17)	100% (7/7)	83% (5/6)	90% (27/30)
	With NS3 D168 RAS	50% (1/2)	25% (1/4)	1/1	43% (3/7)
Baseline NS5A Q30 RAS					
NS5A I-Exp, NS/4A PI-Naïve	No NS5A Q30 RAS	100% (7/7)	100% (9/9)	100% (3/3)	100% (19/19)
	With NS5A Q30 RAS	75% (6/8)	75% (3/4)	1/1	77% (10/13)
NS3/4A PI- and NS5A I-Exp	No NS5A Q30 RAS	100% (10/10)	83% (5/6)	50% (1/2)	89% (16/18)
	With NS5A Q30 RAS	67% (6/9)	60% (3/5)	100% (5/5)	74% (14/19)
Baseline NS3 D168 OR NS5A Q30 RAS					
NS5A I-Exp, NS/4A PI-Naïve	No NS3 D168 OR NS5A Q30 RAS	100% (7/7)	100% (9/9)	100% (3/3)	100% (19/19)
	With NS3 D168 OR NS5A Q30 RAS	75% (6/8)	75% (3/4)	1/1	77% (10/13)
NS3/4A PI- and NS5A I-Exp	No NS3 D168 OR NS5A Q30 RAS	100% (9/9)	100% (5/5)	50% (1/2)	94% (15/16)
	With NS3 D168 OR NS5A Q30 RAS	70% (7/10)	50% (3/6)	100% (5/5)	71% (15/21)
Baseline NS5A RAS (Substitution(s) at any NS5A resistance-associated position)					
NS5A I-Exp, NS/4A PI-Naïve	No NS5A RAS	100% (2/2)	100% (4/4)	1/1	100% (7/7)
	With NS5A RAS	85% (11/13)	89% (8/9)	100% (3/3)	88% (22/25)
	1 NS5A RAS	80% (4/5)	100% (4/4)	100% (2/2)	91% (10/11)
	≥2 NS5A RAS	88% (7/8)	80% (4/5)	1/1	86% (12/14)
NS3/4A PI- and NS5A I-Exp	No NS5A RAS	100% (4/4)	100% (3/3)	1/1	100% (8/8)
	With NS5A RAS	80% (12/15)	63% (5/8)	83% (5/6)	76% (22/29)
	1 NS5A RAS	89% (8/9)	100% (3/3)	100% (3/3)	93% (14/15)
	≥2 NS5A RAS	67% (4/6)	40% (2/5)	67% (2/3)	57% (8/14)
Baseline NS3 D168 OR NS5A Q30 OR ≥2 NS5A RAS (15%)					
NS5A I-Exp, NS/4A PI-Naïve	No NS3 D168, OR NS5A Q30, OR ≥2 NS5A RAS	100% (5/5)	100% (8/8)	100% (3/3)	100% (16/16)
	With NS3 D168, OR NS5A Q30, OR ≥2 NS5A RAS	80% (8/10)	80% (4/5)	1/1	81% (13/16)
NS3/4A PI- and NS5A I-Exp	No NS3 D168, OR NS5A Q30, OR ≥2 NS5A RAS	100% (8/8)	100% (4/4)	1/1	100% (13/13)
	With NS3 D168, OR NS5A Q30, OR ≥2 NS5A RAS	73% (8/11)	57% (4/7)	83% (5/6)	71% (17/24)

HCV GT4 Infected Subjects

Only 4 subjects with HCV GT4 infection were enrolled in MAGELLAN-1. Baseline disease and resistance characteristics are summarized in Table 80 (FDA analysis). All 4 subjects achieved SVR12. No subjects had a reported 4a subtype, which is the most common GT4 subtype in the U.S. Due to the limited number of subjects it is not possible to determine if the proposed GLE/PIB treatment regimens are optimal for (b) (4)

(b) (4)

Table 80. Baseline disease and resistance characteristics of HCV GT4 infected subjects enrolled in MAGELLAN-1. RAS data are based on the NGS 15% sensitivity cutoff. *investigational DAA

USUBJID	GT4 subtype	Prior DAA Class Exp	Prior DAA Experience			BL RAS (ref:QC384)		Cirr?	Regimen (Arm)
			NS3 PI	NS5A-I	Other	NS3	NS5A		
M15410-44318-3620	4A/C/D (unkn.)	PI-Exp, NS5A-I Naïve	SMV		SOF	ND	ND	Y	GLE/PIB 16W (E)
M15410-44318-3624	4R	NS5A I-Exp, PI-Naïve		LDV	SOF	none	I28V, L31M	Y	GLE/PIB 16W (E)
M15410-37759-4522	4R	PI- and NS5A I-Exp	SMV	LDV (b) (4)	SOF	R155K	I28V, L31V	N	GLE/PIB 12W (D)
M15410-44318-3622	4R	PI- and NS5A I-Exp	PTV/r	OBV	DSV	none	I28C, P58S	N	GLE/PIB 16W (E)

Discussion of treatment recommendations for GT1, NS5A inhibitor-experienced patients

A key review issue related to the MAGELLAN-1 trial was whether the sponsor had sufficient evidence to support a treatment recommendation for HCV GT1 infected, NS5A inhibitor-experienced patients. Based on extensive discussion within the Division and with the sponsor, the Division ultimately concluded that the data from MAGELLAN-1 support a treatment recommendation for GLE/PIB for 16 weeks for HCV GT1 infected, NS5A inhibitor-experienced, NS3/4A PI-naïve patients. However, the data were not supportive for a treatment recommendation for GT1 infected patients with experience to both NS5A inhibitors and NS3/4A PIs due to higher rates of treatment failure in this subpopulation.

In support of GLE/PIB for 16 weeks for HCV GT1 infected, NS5A inhibitor-experienced, NS3/4A PI-naïve patients, this patient subgroup achieved a favorable 16/17 (94%) SVR12 rate in MAGELLAN-1, Arm E. Furthermore, favorable efficacy data from subjects who received GLE/PIB for 12 weeks would also support the 16-week treatment recommendation, as any subject who achieved SVR12 with GLE/PIB for 12 weeks would have achieved SVR12 with GLE/PIB for 16 weeks. The pooled GLE/PIB 12-week + 16-week SVR12 rate for GT1, NS5A inhibitor-experienced, NS3/4A PI-naïve subjects was 34/37 (92%).

To provide further evidence that GLE/PIB for 16 weeks is effective for GT1, NS5A inhibitor-experienced, NS3/4A PI-naïve patients, the sponsor also considered NS5A inhibitor-experienced subjects who were NS3/4A PI-experienced, but who did not have detected baseline NS3 RASs at positions R155, A156 or D168. The rationale behind this analysis is that NS5A inhibitor-experienced, NS3/4A PI-experienced subjects without these RASs at baseline would be clinically similar to NS5A inhibitor-experienced subjects who never received an NS3/4A PI. This reviewer agrees that this approach could be used to increase the confidence that GLE/PIB for 16 weeks is effective for GT1, NS5A inhibitor-experienced, NS3/4A PI-naïve subjects, as these baseline NS3 RASs are rarely detected in patients without prior NS3/4A PI experience. However, NS3 position R155 is not a major GLE resistance-associated position, and no subjects in MAGELLAN-1 had a baseline NS3 A156 RAS. Therefore, this reviewer considered only NS3 position D168, and only GT1a subjects, as only 1 HCV GT1b infected subject experienced virologic failure in the trial. Indeed, HCV GT1a infected, NS5A inhibitor-

experienced, NS3/4A PI-experienced subjects without baseline D168 RASs achieved a favorable SVR12 rate of 27/30 (90%) across all arms of MAGELLAN-1 (see Table 79 above), providing further confidence that GLE/PIB for 16 weeks is reasonably effective for GT1, NS5A inhibitor-experienced, NS3/4A PI-naïve patients.

While the review team agreed to include a treatment recommendation of GLE/PIB for 16 weeks for GT1, NS5A inhibitor-experienced, NS3/4A PI-naïve patients, the team concluded that there were insufficient data to include an (b) (4) recommendation in Section 2 of the prescribing information. In this subpopulation in MAGELLAN-1, only one subject overall experienced virologic failure with GLE/PIB for 16 weeks, and only 4 HCV GT1a subjects had a baseline NS5A Q30 RAS (3/4 achieved SVR12). Nevertheless, key baseline and treatment-emergent resistance analysis results from MAGELLAN-1 should be described in Section 12.4 of the prescribing information so that clinicians can consider the need for baseline resistance testing on a case-by-case basis. (b) (4) .

For additional details on this review issue, see the internal Midcycle-related discussion slides in Appendix I.

7. CONCLUSIONS

This Original NDA is approvable from a Clinical Virology perspective, pending final agreement on the prescribing information.

8. PRESCRIBING INFORMATION (LABEL)

8.1 Proposed Prescribing Information (with initial Reviewer-recommended changes)

Clinical virology-related sections of the proposed Mavyret™ prescribing information and suggested edits are shown below. Note that at the time of finalization of this review the review team was still negotiating prescribing information language with the sponsor, so the edits shown below should be considered preliminary.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Mechanism of Action

TRADENAME is a fixed-dose combination of glecaprevir and pibrentasvir, (b) (4) which are direct-acting antiviral agents against the hepatitis C virus (b) (4) (b) (4) (b) (4) *[see Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Glecaprevir

Glecaprevir is an (b) (4) inhibitor of the HCV NS3/4A protease, which is necessary for the proteolytic cleavage of the HCV encoded polyprotein (into mature forms of the NS3, NS4A, NS4B, NS5A, and NS5B proteins) and is essential for viral replication. In a biochemical assay, glecaprevir inhibited the proteolytic activity of recombinant NS3/4A enzymes from clinical isolates of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a with IC_{50} values ranging from 3.5 to 11.3 nM.

Pibrentasvir

Pibrentasvir is an (b) (4) inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. The mechanism of action of pibrentasvir has been characterized based on cell culture antiviral activity and drug resistance mapping studies.

Antiviral Activity

(b) (4)

Combination Antiviral Activity (b) (4)

Evaluation of combination of glecaprevir and pibrentasvir showed no antagonism in antiviral activity in HCV genotype 1 replicon cell culture assays.

Resistance

In Cell Culture

(b) (4)

Selection of HCV genotype 1a, 1b, 2a, 3a, 4a or 6a replicons for reduced susceptibility to glecaprevir resulted in the emergence of amino acid substitutions most commonly at NS3 positions A156 or D/Q168. Individual substitutions at NS3 amino acid position A156 introduced into HCV replicons by site-directed mutagenesis generally caused the greatest reductions (>100-fold) in susceptibility to glecaprevir. Individual substitutions at NS3 position D/Q168 had varying effects on glecaprevir susceptibility depending on HCV genotype/subtype and specific amino acid change, with the greatest reductions (>30-fold) observed in genotypes 1a (D168F/Y), 3a (Q168R) and 6a (D168A/G/H/V/Y). Combinations of NS3 Y56H plus D/Q168 substitutions often resulted in greater reductions in glecaprevir susceptibility. An NS3 Q80R substitution in genotype 3a caused a 21-fold reduction in glecaprevir susceptibility, while Q80 substitutions in genotypes 1a and 1b (including genotype 1a Q80K) did not reduce glecaprevir susceptibility. Individual amino acid Ssubstitutions associated with resistance to other (b) (4) (b) (4) HCV protease inhibitors (b) (4) at positions 36, 43, 54, 55, 56, 155, 166, or 170 in NS3 (b) (4) (b) (4) enerally did not reduce susceptibility to glecaprevir- (b) (4) (b) (4)

Selection of HCV genotype 1a, 2a or 3a replicons for reduced susceptibility to pibrentasvir resulted in the emergence of amino acid substitutions at known NS5A inhibitor resistance-associated positions, including Q30D/deletion, Y93D/H/N or H58D +Y93H in genotype 1a replicons, F28S + M31I or P29S + K30G in genotype 2a replicons, and Y93H in genotype 3a replicons. (b) (4) (b) (4)

In Clinical Studies

Studies in Treatment-Naïve and Peginterferon, Ribavirin and/or Sofosbuvir Treatment-Experienced Subjects with or without Cirrhosis

In pooled analyses of NS3/4A protease inhibitor- and NS5A inhibitor-naïve subjects who received TRADENAME for 8, 12, or 16 weeks in Phase 2 and 3 clinical studies, treatment-emergent (b) (4) resistance analyses were conducted for 22 subjects whc (b) (4) experienced virologic failure (2 with genotype 1, 2 with genotype 2, 18 with genotype 3 infection). No subjects with HCV genotype 4, 5 or 6 infection experienced virologic failure.

Among the 2 genotype 1-infected subjects who experienced virologic failure, both subjects had a subtype 1a infection. ~~, one~~ One subject had treatment-emergent substitutions A156V in NS3, and Q30R, ~~/~~L31M and~~/~~ H58D in NS5A (Q30R and L31M were also detected at a low frequency at baseline). One ~~, and 1~~ subject had treatment-emergent Q30R~~/~~ and H58D (while Y93N was present at baseline and post-treatment) in NS5A.

Among the 2 genotype 2-infected subjects, both subjects had a subtype 2a infection, and no treatment-emergent substitutions were observed in NS3 or NS5A. (b) (4)

Among the 18 genotype 3-infected subjects (b) (4) who experienced virologic failure, treatment-emergent NS3 substitutions Y56H/N, Q80K/R, A156G, or Q168L/R were observed in 11 subjects. A166S or Q168R were present at baseline and post-treatment in 5 subjects. Treatment-emergent NS5A substitutions M28G, A30G/K, L31F, P58T, or Y93H were observed in 16 subjects, and 13 subjects had A30K (n=9) or Y93H (n=5) at baseline and post-treatment.

Studies in Subjects with or without Cirrhosis who were Treatment-Experienced to NS3/4A Protease and/or NS5A Inhibitors

~~Ten of 113~~ Treatment-emergent resistance analyses were conducted for 11 HCV genotype 1 infected subjects (10 genotype 1a, 1 genotype 1b) with prior NS3/4A protease inhibitor or NS5A inhibitor treatment experience who experienced virologic failure ~~treated~~ with TRADENAME with or without ribavirin in the MAGELLAN-1 study (b) (4) (b) (4)

(b) (4) ~~t~~Treatment-emergent NS3 substitutions V36A/M, Y56H, R155K/T, A156G/T/V, or D168A/T were observed in 73% ((b) (4) 8/11) of subjects. (b) (4) Nine of 10 subjects (90%, not including one subject missing NS5A data at failure) (b) (4) had treatment-emergent NS5A substitutions M28A/G (or L28M for genotype 1b), P29Q/R, Q30K/R, H58D or Y93H/N. (b) (4) (b) (4) (b) (4) All 11 subjects also had NS5A inhibitor resistance-associated substitutions detected at baseline, and 7/11 had NS3 protease inhibitor resistance-associated substitutions detected at baseline (see Cross-Resistance for the effect of baseline resistance-associated substitutions on treatment response for NS3/4A protease inhibitor or NS5A inhibitor treatment-experienced patients).

Effect of Baseline HCV Amino Acid Polymorphisms on Treatment Response (NS3/4A Protease Inhibitor- and NS5A Inhibitor-Naïve Subjects)

A pooled analysis of NS3/4A protease inhibitor- and NS5A inhibitor-naïve subjects [redacted] who received TRADENAME in the Phase 2 and Phase 3 clinical studies was conducted to identify the HCV subtypes represented and explore the association between baseline amino acid polymorphisms and treatment outcome [redacted]. Baseline polymorphisms relative to a subtype-specific reference sequence at resistance-associated amino acid positions 155, 156, and 168 in NS3, and 24, 28, 30, 31, 58, 92, and 93 in NS5A were evaluated at a 15% detection threshold by next-generation sequencing. Among subjects who received TRADENAME for 8-, 12-, or 16 weeks, ~~B~~baseline polymorphisms in NS3 were detected in 1[redacted]% (9/845), [redacted]1% (3/398), [redacted]2% (10/613), 1[redacted]% (2/164), 42[redacted]% (13/31), and [redacted]3% (1/34) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively. No baseline polymorphisms were detected at NS3 amino acid position 156 across all genotypes. Baseline polymorphisms in NS5A were detected in 27[redacted]% (225/841), [redacted]30% (331/415), 22[redacted]% (136/615), [redacted]50% (80/161), [redacted]13-% (4/31), and 54[redacted]% (20/37) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively.

Genotype 1, 2, 4, 5, and 6: Baseline HCV polymorphisms in genotypes 1, 2, 4, 5 and 6 had no impact on treatment outcome.

Genotype 3: Among [redacted]-treatment-naïve, genotype 3-infected subjects without cirrhosis who received TRADENAME for 8 weeks, an NS5A A30K polymorphism was detected in 10% (18/181) of subjects, of whom 78% (14/18) achieved SVR12. Insufficient data are available to characterize the impact of the A30K polymorphism in genotype 3-infected subjects with cirrhosis (n=1, SVR12) or prior treatment experience (n=1, relapse) who received~~receiving the outcome~~. All genotype 3-infected subjects [redacted] (100%, [redacted]16/[redacted]16) with Y93H in NS5A at baseline who received recommended TRADENAME regimens achieved SVR12.

[redacted]

Cross-resistance

[redacted]

[redacted]Based on resistance patterns observed in cell culture replicon studies and HCV-infected subjects, cross-resistance is possible between glecaprevir and other HCV NS3/4A protease inhibitors, and between pibrentasvir and other HCV NS5A inhibitors. Cross-resistance is not expected between TRADENAME and sofosbuvir, pegylated interferon or ribavirin.

In the MAGELLAN--1 study, HCV genotype 1-infected subjects who had failed prior treatment with NS3/4A protease and/or NS5A inhibitors were treated with TRADENAME for 12 or 16 weeks. Baseline sequences were analyzed by next generation sequencing at a 15% detection threshold. (b) (4)

Among 23 NS3/4A protease inhibitor~~PI~~-experienced/NS5A inhibitor-naïve subjects (b) (4) who received TRADENAME for 12 weeks in MAGELLAN-1 ((b) (4) 2 non-virologic failure subjects), 2 subjects each had baseline NS3 R155K or D168V/E substitutions (b) (4) all 23 subjects achieved SVR12.

Among (b) (4) NS5A inhibitor-experienced/ (b) (4) protease inhibitor~~PI~~-naïve subjects~~)~~ (b) (4) who received TRADENAME for 16 weeks (b) (4) baseline NS5A resistance-associated substitutions were detected in 73% (11/15) of subjects with available data, of whom 91% (10/11) achieved SVR12-. The non-SVR12 subject experienced on-treatment virologic failure and had a genotype 1a infection with baseline NS5A Q30R and L31M substitutions. (b) (4)

Persistence of Resistance-Associated Substitutions

Data on the persistence of glecaprevir and pibrentasvir resistance-associated substitutions are not available. NS5A resistance-associated substitutions observed in patients treated with other NS5A inhibitors have been found to persist for longer than 1 year. In patients treated with other NS3/4A protease inhibitors, viral populations with NS3 resistance-associated substitutions (b) (4) (b) (4) have been found to decline (b) (4) in some patients through post-treatment weeks 24 and 48. (b) (4) (b) (4) The long-term clinical impact of the emergence or persistence of virus containing glecaprevir or pibrentasvir resistance-associated substitutions is unknown.

8.2 Reviewer's Proposed Prescribing Information (clean)

12.1 Mechanism of Action

Mechanism of Action

TRADENAME is a fixed-dose combination of glecaprevir and pibrentasvir, which are direct-acting antiviral agents against the hepatitis C virus *[see Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Glecaprevir

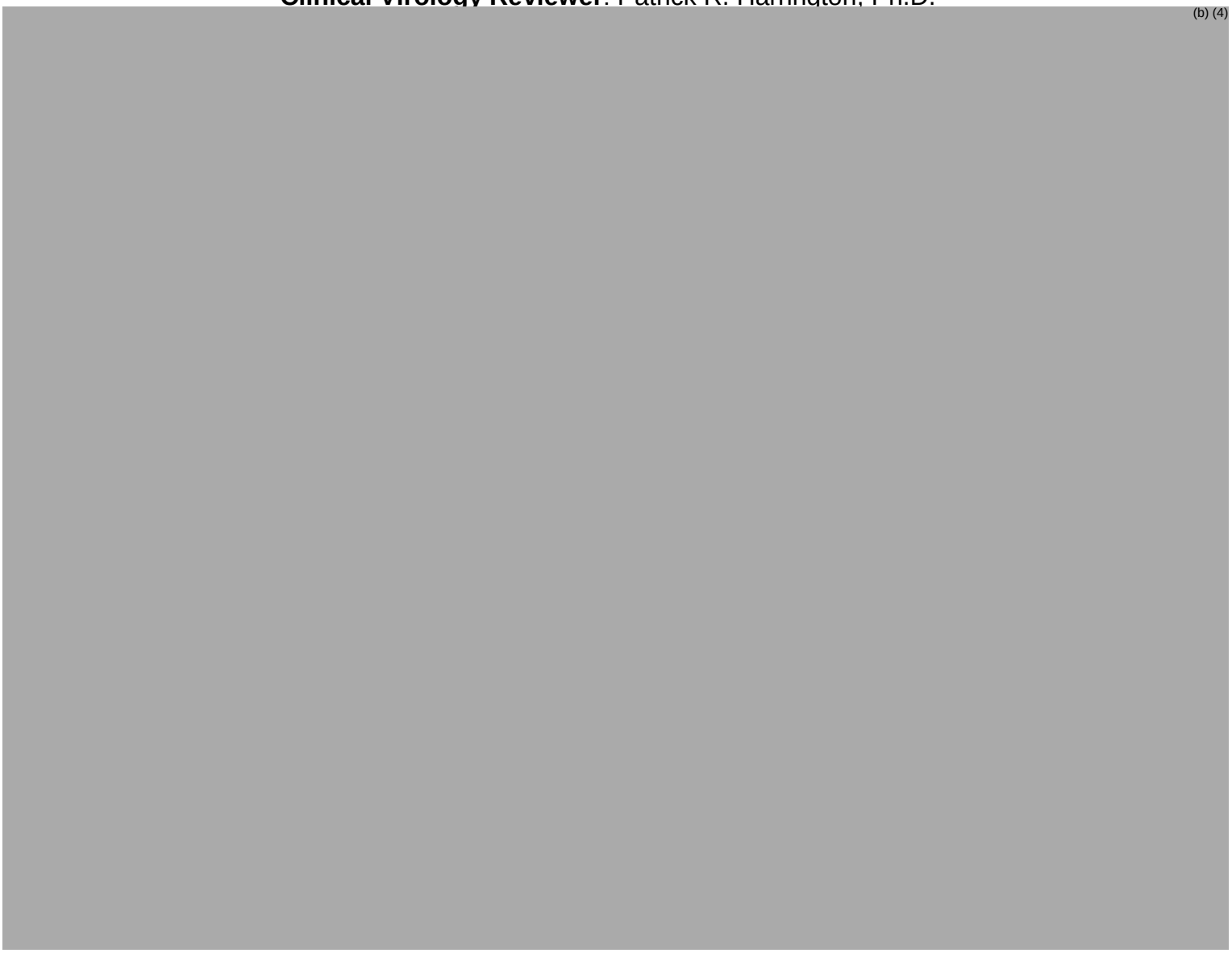
Glecaprevir is an inhibitor of the HCV NS3/4A protease, which is necessary for the proteolytic cleavage of the HCV encoded polyprotein (into mature forms of the NS3, NS4A, NS4B, NS5A, and NS5B proteins) and is essential for viral replication. In a biochemical assay, glecaprevir inhibited the proteolytic activity of recombinant NS3/4A enzymes from clinical isolates of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a with IC_{50} values ranging from 3.5 to 11.3 nM.

Pibrentasvir

Pibrentasvir is an inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. The mechanism of action of pibrentasvir has been characterized based on cell culture antiviral activity and drug resistance mapping studies.

Antiviral Activity

(b) (4)



Combination Antiviral Activity

Evaluation of combination of glecaprevir and pibrentasvir showed no antagonism in antiviral activity in HCV genotype 1 replicon cell culture assays.

Resistance

In Cell Culture

Selection of HCV genotype 1a, 1b, 2a, 3a, 4a or 6a replicons for reduced susceptibility to glecaprevir resulted in the emergence of amino acid substitutions most commonly at NS3 positions A156 or D/Q168. Individual substitutions at NS3 amino acid position A156 introduced into HCV replicons by site-directed mutagenesis generally caused the greatest reductions (>100-fold) in susceptibility to glecaprevir. Individual substitutions at NS3 position D/Q168 had varying effects on glecaprevir susceptibility depending on HCV genotype/subtype and specific amino acid change, with the greatest reductions (>30-fold) observed in genotypes 1a (D168F/Y), 3a (Q168R) and 6a (D168A/G/H/V/Y). Combinations of NS3 Y56H plus D/Q168 substitutions often resulted

in greater reductions in glecaprevir susceptibility. An NS3 Q80R substitution in genotype 3a caused a 21-fold reduction in glecaprevir susceptibility, while Q80 substitutions in genotypes 1a and 1b (including genotype 1a Q80K) did not reduce glecaprevir susceptibility. Individual amino acid substitutions associated with resistance to other HCV protease inhibitors at positions 36, 43, 54, 55, 56, 155, 166, or 170 in NS3 generally did not reduce susceptibility to glecaprevir.

Selection of HCV genotype 1a, 2a or 3a replicons for reduced susceptibility to pibrentasvir resulted in the emergence of amino acid substitutions at known NS5A inhibitor resistance-associated positions, including Q30D/deletion, Y93D/H/N or H58D +Y93H in genotype 1a replicons, F28S + M31I or P29S + K30G in genotype 2a replicons, and Y93H in genotype 3a replicons. (b) (4)

(b) (4)

In Clinical Studies

Studies in Treatment-Naïve and Peginterferon, Ribavirin and/or Sofosbuvir Treatment-Experienced Subjects with or without Cirrhosis

In pooled analyses of NS3/4A protease inhibitor- and NS5A inhibitor-naïve subjects who received TRADENAME for 8, 12, or 16 weeks in Phase 2 and 3 clinical studies, treatment-emergent resistance analyses were conducted for 22 subjects who experienced virologic failure (2 with genotype 1, 2 with genotype 2, 18 with genotype 3 infection). No subjects with HCV genotype 4, 5 or 6 infection experienced virologic failure.

Among the 2 genotype 1-infected subjects who experienced virologic failure, both subjects had a subtype 1a infection. One subject had treatment-emergent substitutions A156V in NS3, and Q30R, L31M and H58D in NS5A (Q30R and L31M were also detected at a low frequency at baseline). One subject had treatment-emergent Q30R and H58D (while Y93N was present at baseline and post-treatment) in NS5A.

Among the 2 genotype 2-infected subjects, both subjects had a subtype 2a infection, and no treatment-emergent substitutions were observed in NS3 or NS5A.

Among the 18 genotype 3-infected subjects who experienced virologic failure, treatment-emergent NS3 substitutions Y56H/N, Q80K/R, A156G, or Q168L/R were observed in 11 subjects. A166S or Q168R were present at baseline and post-treatment in 5 subjects. Treatment-emergent NS5A substitutions M28G, A30G/K, L31F, P58T, or Y93H were observed in 16 subjects, and 13 subjects had A30K (n=9) or Y93H (n=5) at baseline and post-treatment.

Studies in Subjects with or without Cirrhosis who were Treatment-Experienced to NS3/4A Protease and/or NS5A Inhibitors

Treatment-emergent resistance analyses were conducted for 11 HCV genotype 1 infected subjects (10 genotype 1a, 1 genotype 1b) with prior NS3/4A protease inhibitor or NS5A inhibitor treatment experience who experienced virologic failure with TRADENAME with or without ribavirin in the MAGELLAN-1 study. Treatment-emergent NS3 substitutions V36A/M, Y56H, R155K/T, A156G/T/V, or D168A/T were observed in 73% (8/11) of subjects. Nine of 10 subjects (90%, not including one subject missing NS5A data at failure) had treatment-emergent NS5A substitutions M28A/G (or L28M for genotype 1b), P29Q/R, Q30K/R, H58D or Y93H/N. All 11 subjects also had NS5A inhibitor resistance-associated substitutions detected at baseline, and

7/11 had NS3 protease inhibitor resistance-associated substitutions detected at baseline (see Cross-Resistance for the effect of baseline resistance-associated substitutions on treatment response for NS3/4A protease inhibitor or NS5A inhibitor treatment-experienced patients).

Effect of Baseline HCV Amino Acid Polymorphisms on Treatment Response (NS3/4A Protease Inhibitor- and NS5A Inhibitor-Naïve Subjects)

A pooled analysis of NS3/4A protease inhibitor- and NS5A inhibitor-naïve subjects who received TRADENAME in the Phase 2 and Phase 3 clinical studies was conducted to identify the HCV subtypes represented and explore the association between baseline amino acid polymorphisms and treatment outcome . Baseline polymorphisms relative to a subtype-specific reference sequence at resistance-associated amino acid positions 155, 156, and 168 in NS3, and 24, 28, 30, 31, 58, 92, and 93 in NS5A were evaluated at a 15% detection threshold by next-generation sequencing. Among subjects who received TRADENAME for 8-, 12-, or 16 weeks, baseline polymorphisms in NS3 were detected in 1% (9/845), 1% (3/398), 2% (10/613), 1% (2/164), 42% (13/31), and 3% (1/34) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively. No baseline polymorphisms were detected at NS3 amino acid position 156 across all genotypes. Baseline polymorphisms in NS5A were detected in 27% (225/841), 80% (331/415), 22% (136/615), 50% (80/161), 13% (4/31), and 54% (20/37) of subjects with HCV genotype 1, 2, 3, 4, 5, and 6 infection, respectively.

Genotype 1, 2, 4, 5, and 6: Baseline HCV polymorphisms in genotypes 1, 2, 4, 5 and 6 had no impact on treatment outcome.

Genotype 3: Among treatment-naïve, genotype 3-infected subjects without cirrhosis who received TRADENAME for 8 weeks, an NS5A A30K polymorphism was detected in 10% (18/181) of subjects, of whom 78% (14/18) achieved SVR12. Insufficient data are available to characterize the impact of the A30K polymorphism in genotype 3-infected subjects with cirrhosis (n=1, SVR12) or prior treatment experience (n=1, relapse) who received recommended TRADENAME regimens. All genotype 3-infected subjects (100%, 16/16) with Y93H in NS5A at baseline who received recommended TRADENAME regimens achieved SVR12.

Cross-resistance

Based on resistance patterns observed in cell culture replicon studies and HCV-infected subjects, cross-resistance is possible between glecaprevir and other HCV NS3/4A protease inhibitors, and between pibrentasvir and other HCV NS5A inhibitors. Cross-resistance is not expected between TRADENAME and sofosbuvir, pegylated interferon or ribavirin.

In the MAGELLAN-1 study, HCV genotype 1-infected subjects who had failed prior treatment with NS3/4A protease and/or NS5A inhibitors were treated with TRADENAME for 12 or 16 weeks. Baseline sequences were analyzed by next generation sequencing at a 15% detection threshold.

Among 23 NS3/4A protease inhibitor-experienced/NS5A inhibitor-naïve subjects who received TRADENAME for 12 weeks in MAGELLAN-1 (censoring 2 non-virologic failure subjects), 2 subjects each had baseline NS3 R155K or D168V/E substitutions; all 23 subjects achieved SVR12.

Among NS5A inhibitor-experienced/protease inhibitor-naïve subjects who received TRADENAME for 16 weeks, baseline NS5A resistance-associated substitutions were detected in 73% (11/15) of subjects with available data, of whom 91% (10/11) achieved SVR12. The non-SVR12 subject experienced on-treatment virologic failure and had a genotype 1a infection with baseline NS5A Q30R and L31M substitutions. (b) (4)

(b) (4)

(b) (4)

Persistence of Resistance-Associated Substitutions

Data on the persistence of glecaprevir and pibrentasvir resistance-associated substitutions are not available. NS5A resistance-associated substitutions observed in patients treated with other NS5A inhibitors have been found to persist for longer than 1 year. In patients treated with other NS3/4A protease inhibitors, viral populations with NS3 resistance-associated substitutions have been found to decline (b) (4) in some patients through post-treatment weeks 24 and 48. The long-term clinical impact of the emergence or persistence of virus containing glecaprevir or pibrentasvir resistance-associated substitutions is unknown.

8.3 Final Approved Package Insert

Due to the timing of NDA milestones and PDUFA goal deadlines, the final approved package insert was not available at the time of finalization of this review.

9. RECOMMENDATIONS

This reviewer recommends the following post-marketing commitments or requirements (as appropriate):

(b) (4)

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11. APPENDICES

Appendix A: Site-directed mutant replicon phenotype data for glecaprevir (GLE)

Replication capacity (RC) is not shown for chimeric replicons. Highlight indicates EC_{50} fold-changes (FC) ≥10 relative to subtype parent replicons (arbitrary cutoff). NA, data not available due to low replication capacity.

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
1a	Wild Type (H77)	11	0.21 ± 0.08	0.77 ± 0.24	-	100
1a	V36A	3	0.18 ± 0.01	1.1 ± 0.16	0.8	130
1a	V36L	3	0.16 ± 0.02	1.2 ± 0.10	0.8	82
1a	V36M	8	0.28 ± 0.10	1.1 ± 0.31	1.4	81
1a	Q41R	3	0.33 ± 0.07	1.7 ± 0.75	1.6	36
1a	F43L	3	0.05 ± 0.01	0.27 ± 0.03	0.3	17
1a	T54S	3	0.20 ± 0.06	0.79 ± 0.22	1.0	6.2
1a	V55I	3	0.05 ± 0.01	0.29 ± 0.02	0.2	81
1a	Y56H	9	0.21 ± 0.06	1.9 ± 0.74	1.0	3.5
1a	V71A	4	0.09 ± 0.03	0.62 ± 0.09	0.4	1.4
1a	Q80K	6	0.19 ± 0.05	0.65 ± 0.06	0.9	91
1a	Q80L	5	0.44 ± 0.33	1.5 ± 0.82	2.1	38
1a	Q80R	3	0.27 ± 0.15	1.2 ± 0.26	1.3	44
1a	Q89R	3	0.34 ± 0.10	1.5 ± 0.50	1.6	105
1a	A150V	3	0.10 ± 0.01	0.56 ± 0.16	0.5	66
1a	R155G	3	NA	NA	-	2.0
1a	R155K	14	0.11 ± 0.03	0.48 ± 0.15	0.5	31
1a	R155M	3	0.18 ± 0.02	0.86 ± 0.16	0.9	5.6
1a	R155S	3	0.34 ± 0.12	2.4 ± 0.32	1.6	2.1
1a	R155T	3	0.39 ± 0.14	2.0 ± 0.71	1.9	5.1
1a	R155V	3	0.21 ± 0.06	1.8 ± 0.30	1.0	20
1a	A156G	3	0.13 ± 0.03	1.1 ± 0.03	0.6	45
1a	A156T	6	286 ± 92.8	1527 ± 380	1361	5.2
1a	A156V	5	NA	NA	-	< 0.5
1a	D168A	3	0.84 ± 0.45	7.5 ± 6.8	4.0	35
1a	D168E	6	0.27 ± 0.09	1.7 ± 0.83	1.3	34
1a	D168F	3	11.5 ± 0.18	18.9 ± 1.4	55	4.0
1a	D168H	3	0.91 ± 0.21	7.0 ± 1.8	4.3	24
1a	D168N	3	0.08 ± 0.01	0.60 ± 0.04	0.4	28
1a	D168V	4	0.93 ± 0.28	8.8 ± 2.9	4.4	1.5
1a	D168Y	10	8.6 ± 3.5	24.8 ± 10.1	41	3.5
1a	I170T	3	0.10 ± 0.02	0.60 ± 0.07	0.5	9.9
1a	I170V	3	0.21 ± 0.03	1.1 ± 0.25	1.0	77
1a	P334S	4	0.12 ± 0.02	0.65 ± 0.07	0.6	97
1a	S342P	4	0.10 ± 0.03	0.70 ± 0.10	0.5	52
1a	E357K	3	0.34 ± 0.00	1.1 ± 0.10	1.6	131
1a	V406A	4	0.17 ± 0.03	0.70 ± 0.09	0.8	97
1a	V406I	3	0.14 ± 0.05	0.85 ± 0.33	0.7	92
1a	T449I	3	0.16 ± 0.01	0.51 ± 0.07	0.8	85
1a	L470S	4	NA	NA	-	< 0.5
1a	V23A (NS4A)	4	0.36 ± 0.07	1.8 ± 0.47	1.7	70
1a	V36M + R155K	3	0.14 ± 0.03	0.60 ± 0.01	0.7	29
1a	Q41R + Q89R	3	0.31 ± 0.06	3.3 ± 1.9	1.5	22
1a	Q41R + I170V	7	NA	NA	-	< 0.5
1a	Q41R + D168E	3	1.7 ± 0.14	7.2 ± 0.95	8.0	154
1a	F43L + R155K	3	0.16 ± 0.01	0.91 ± 0.03	0.8	7.2
1a	F43L + D168V	3	2.7 ± 0.46	37.4 ± 2.2	13	23
1a	Y56H + A156G	3	NA	NA	-	< 0.5
1a	Y56H + D168A	3	8.2 ± 1.8	33.3 ± 3.8	39	46
1a	Y56H + D168E	3	9.8 ± 0.72	33.9 ± 9.3	47	4.7
1a	Y56H + D168V	3	8.9 ± 1.2	46.8 ± 18.6	42	15
1a	Y56H + D168Y	3	9.4 ± 3.7	34.8 ± 13.1	45	1.1
1a	V71A + I170V	3	0.69 ± 0.12	3.4 ± 1.1	3.3	1.0

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
1a	Q80K + R155G	3	1.2 ± 0.47	7.4 ± 2.1	5.8	2.2
1a	Q80K + R155K	3	0.09 ± 0.03	0.42 ± 0.05	0.4	77
1a	Q80K + D168A	3	0.78 ± 0.04	5.2 ± 0.29	3.7	28
1a	Q80K + D168V	3	1.4 ± 0.12	16.9 ± 2.0	6.9	5.0
1a	Q80K + D168Y	3	10.0 ± 4.1	51.6 ± 16.9	48	2.0
1a	Q89R + A156T	3	753 ± 30.9	>10000	3585	1.0
1a	Q89R + A156V	3	2294 ± 241	>10000	10922	2.0
1a	A150V + A156T	3	NA	NA	-	< 0.5
1a	R155K + P334S	3	0.06 ± 0.02	0.36 ± 0.13	0.3	68
1a	R155K + S342P	3	0.06 ± 0.03	0.37 ± 0.11	0.3	20
1a	R155K + V406A	3	0.08 ± 0.02	0.46 ± 0.13	0.4	64
1a	R155K + T449I	3	0.08 ± 0.03	0.39 ± 0.08	0.4	86
1a	R155T + D168N	3	5.9 ± 0.71	28.5 ± 12.1	28	52
1a	A156G + D168A	3	6.7 ± 2.2	32.6 ± 3.0	32	9.1
1a	I170T + D168E	3	0.25 ± 0.05	1.4 ± 0.57	1.2	21
1a	I170T + D168N	3	0.21 ± 0.02	2.5 ± 0.37	1.0	5.3
1a	I170V + D168E	3	0.18 ± 0.01	0.87 ± 0.04	0.9	22
1a	I170V + D168N	3	0.09 ± 0.02	0.43 ± 0.12	0.4	26
1a	D168V + P334S	3	1.1 ± 0.39	5.3 ± 0.34	5.1	12
1a	D168V + S342P	4	0.68 ± 0.14	6.8 ± 1.9	3.2	28
1a	D168V + E357K	3	6.4 ± 1.9	18.6 ± 0.74	30	24
1a	D168V + V406A	3	1.4 ± 0.47	8.5 ± 1.5	6.7	8.1
1a	D168V + V406I	3	0.49 ± 0.11	11.0 ± 5.8	2.3	13
1a	D168V + L470S	3	NA	NA	-	< 0.5
1a	D168V + T449I	7	0.59 ± 0.25	6.7 ± 1.8	2.8	18
1a	D168Y + P334S	4	9.9 ± 0.92	24.7 ± 9.9	47	1.4
1a	D168Y + S342P	4	5.4 ± 0.79	27.3 ± 8.4	26	11
1a	D168Y + E357K	3	15.1 ± 2.7	21.3 ± 1.1	72	8.2
1a	D168Y + V406A	3	10.9 ± 3.0	32.8 ± 24.4	52	1.5
1a	D168Y + T449I	4	10.6 ± 1.8	27.2 ± 9.7	50	3.3
1a	V23A (NS4A) + D168V	4	NA	NA	-	< 0.5
1a	V23A (NS4A) + D168Y	3	7.1 ± 1.4	61.6 ± 19.6	34	18
1a	V36M + Y56H + D168A	3	33.9 ± 1.3	116 ± 2.4	162	35
1a	V36M + Y56H + D168E	3	26.7 ± 1.6	101 ± 16.7	127	1.6
1a	Y56H + A156G + D168A	3	NA	NA	-	< 0.5
1a	R155K + A156V + D168A	3	NA	NA	-	< 0.5
1b	Wild Type (Con1)	10	0.47 ± 0.13	1.9 ± 0.51	-	100
1b	T54A	3	0.45 ± 0.10	1.9 ± 0.59	1.0	59
1b	T54S	3	NA	NA	-	< 0.5
1b	V55A	3	0.21 ± 0.03	2.2 ± 0.89	0.4	14
1b	Y56F	3	NA	NA	-	1.1
1b	Y56H	6	NA	NA	-	< 0.5
1b	Q80K	3	0.37 ± 0.03	1.1 ± 0.04	0.8	162
1b	Q80L	3	0.30 ± 0.07	1.1 ± 0.10	0.6	123
1b	P89L	4	1.1 ± 0.21	3.7 ± 0.77	2.4	172
1b	R155K	3	0.27 ± 0.11	1.5 ± 0.10	0.6	73
1b	R155Q	3	NA	NA	-	< 0.5
1b	A156S	3	0.20 ± 0.08	2.0 ± 1.1	0.4	61
1b	A156T	6	301 ± 61.6	1545 ± 508	640	19
1b	A156V	7	839 ± 181	3440 ± 547	1786	9.2
1b	D168A	3	0.69 ± 0.11	1.8 ± 0.05	1.5	69
1b	D168E	6	0.40 ± 0.08	1.8 ± 0.55	0.9	80
1b	D168F	3	2.5 ± 0.08	10.3 ± 0.27	5.3	105
1b	D168H	3	0.68 ± 0.05	3.1 ± 0.21	1.4	108
1b	D168K	3	5.3 ± 0.38	20.6 ± 2.0	11	50
1b	D168T	3	1.3 ± 0.20	5.3 ± 2.0	2.8	129
1b	D168V	6	1.5 ± 0.43	5.9 ± 1.5	3.2	157
1b	D168Y	3	0.99 ± 0.11	4.5 ± 0.18	2.1	70
1b	V170A	3	0.49 ± 0.11	2.0 ± 0.64	1.1	64
1b	E176G	3	1.3 ± 0.19	3.3 ± 0.52	2.7	103
1b	Y56F + Q80L	3	0.28 ± 0.02	1.1 ± 0.10	0.6	96
1b	Y56H + D168A	3	8.0 ± 1.5	32.0 ± 6.1	17	6.0
1b	Y56H + D168E	6	NA	NA	-	< 0.5

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
1b	Y56H + D168V	3	7.2 ± 1.3	32.9 ± 3.4	15	22
1b	Y56H + D168Y	3	35.6 ± 4.6	73.1 ± 7.9	76	8.0
1b	Q80K + D168V	3	0.79 ± 0.11	2.9 ± 0.17	1.7	132
1b	P89L + A156T	3	787 ± 115	2451 ± 327	1674	113
1b	P89L + A156V	3	1994 ± 729	5894 ± 1885	4243	119
1b	A156T + D168V	3	643 ± 70.9	3272 ± 218	1368	21
1b	A156V + D168V	3	2465 ± 1321	6167 ± 649	5244	17
1b	A156V + E176G	3	1113 ± 43.3	3108 ± 146	2368	43
2a	Wild Type (JFH-1)	18	2.5 ± 0.69	6.2 ± 1.4	-	100
2a	G15D	3	1.1 ± 0.10	4.0 ± 0.33	0.5	85
2a	V55A	3	2.3 ± 0.32	5.6 ± 0.52	0.9	51
2a	V55I	3	3.1 ± 0.94	4.7 ± 0.91	1.2	43
2a	Y56F	3	1.7 ± 0.66	3.7 ± 0.61	0.7	70
2a	Y56H	6	1.4 ± 0.25	3.8 ± 0.52	0.6	125
2a	A156T	3	541 ± 76.9	957 ± 184	216	69
2a	A156V	3	2857 ± 235	4203 ± 367	1143	43
2a	D168A	6	4.8 ± 1.3	9.3 ± 2.0	1.9	75
2a	D168E	12	8.1 ± 1.9	16.1 ± 3.1	3.3	116
2a	D168H	3	7.9 ± 0.52	18.5 ± 2.5	3.2	86
2a	D168V	3	4.9 ± 0.79	13.2 ± 0.91	2.0	129
2a	D168Y	3	6.0 ± 1.3	17.7 ± 2.9	2.4	107
2a	G15D + A156T	3	871 ± 93.1	1647 ± 329	348	64
2a	G15D + A156V	3	3470 ± 352	5646 ± 906	1388	49
2a	V55A + D168E	3	9.6 ± 1.1	14.6 ± 0.30	3.8	86
2a	V55I + D168E	6	6.6 ± 1.4	16.3 ± 1.9	2.7	69
2a	Y56F + D168E	3	4.0 ± 0.39	7.8 ± 0.91	1.6	160
2a	Y56H + D168A	3	2.3 ± 1.2	7.8 ± 1.2	0.9	85
2a	Y56H + D168E	3	4.6 ± 0.77	14.3 ± 0.88	1.8	61
2a	Y56H + D168V	3	7.5 ± 0.85	28.7 ± 8.2	3.0	100
2b	Wild Type	12	3.1 ± 0.46	8.9 ± 1.4	-	-
2b	Y56F	3	1.8 ± 0.13	8.0 ± 1.2	0.6	-
2b	E79G	3	5.8 ± 0.20	12.5 ± 0.70	1.9	-
2b	P146S	3	4.0 ± 0.26	10.4 ± 1.0	1.3	-
2b	A150V	3	2.4 ± 0.24	8.3 ± 2.0	0.8	-
2b	F154Y	3	2.8 ± 0.61	11.6 ± 1.6	0.9	-
2b	A156M	3	3370 ± 292	4347 ± 653	1087	-
2b	A156T	3	460 ± 172	788 ± 165	148	-
2b	A156V	3	4510 ± 1726	4298 ± 297	1455	-
2b	A160T	3	3.2 ± 0.51	7.9 ± 1.2	1.0	-
2b	D168A	9	3.9 ± 1.0	10.9 ± 2.7	1.3	-
2b	D168E	6	6.6 ± 1.8	13.4 ± 3.7	2.1	-
2b	D168F	3	12.3 ± 1.9	24.4 ± 4.4	4.0	-
2b	D168H	3	8.5 ± 0.06	20.8 ± 2.6	2.7	-
2b	D168S	3	3.8 ± 0.57	8.4 ± 0.96	1.2	-
2b	D168T	3	17.4 ± 1.7	40.2 ± 5.6	5.6	-
2b	D168V	9	9.1 ± 1.2	23.6 ± 4.4	2.9	-
2b	D168Y	3	6.6 ± 1.1	16.7 ± 1.9	2.1	-
2b	E173G	3	7.4 ± 1.7	47.9 ± 1.6	2.4	-
2b	A178T	3	1.9 ± 0.20	5.7 ± 0.44	0.6	-
2b	A178V	3	3.0 ± 0.15	11.0 ± 1.4	1.0	-
2b	Y56F + D168A	3	2.3 ± 0.24	6.4 ± 0.58	0.8	-
2b	Y56F + D168E	3	3.4 ± 2.6	6.8 ± 4.4	1.1	-
2b	Y56F + D168V	3	8.1 ± 0.66	19.7 ± 2.6	2.6	-
2b	Y56H + D168E	3	1.1 ± 0.02	2.8 ± 0.29	0.4	-
2b	E79G + F154Y	3	20.9 ± 2.9	47.9 ± 1.6	6.7	-
2b	F154Y + E173G	3	19.1 ± 4.9	47.5 ± 5.5	6.1	-
2b	A160T + D168V	3	17.1 ± 2.2	37.6 ± 4.5	5.5	-
2b	A160T + D168Y	3	12.3 ± 1.3	22.6 ± 3.8	4.0	-
2b	D168V + A178T	3	13.0 ± 1.6	24.7 ± 3.5	4.2	-
2b	D168V + A178V	3	17.7 ± 3.4	31.2 ± 3.8	5.7	-
3a	Wild Type	10	0.55 ± 0.17	7.2 ± 2.8	-	-
3a	Y56H	3	NA	NA	-	-
3a	Q80R	3	11.5 ± 1.6	296 ± 122	21	-

Subtype	NS3 Amino Acid Substitutions	N	Mean GLE EC_{50} ± SD (nM)	Mean GLE EC_{90} ± SD (nM)	FC EC_{50}	RC (%)
3a	R155K	3	0.28 ± 0.03	2.7 ± 1.1	0.5	-
3a	A156G	3	909 ± 349	3245 ± 463	1654	-
3a	S166A	2	NA	NA	-	-
3a	S166T	8	2.6 ± 0.58	75.5 ± 40.9	4.7	-
3a	Q168H	3	0.40 ± 0.07	9.0 ± 4.2	0.7	-
3a	Q168K	3	NA	NA	-	-
3a	Q168L	3	6.9 ± 1.7	211 ± 178	13	-
3a	Q168R	3	30.0 ± 10.4	305 ± 141	54	-
3a	Y56H + A156G	3	NA	NA	-	-
3a	Y56H + S166A	2	NA	NA	-	-
3a	Y56H + S166T	3	NA	NA	-	-
3a	Y56H + Q168K	3	NA	NA	-	-
3a	Y56H + Q168L	6	NA	NA	-	-
3a	Y56H + Q168R	3	763 ± 363	4259 ± 1280	1387	-
3a	Q80R + S166T	3	29.8 ± 2.4	904 ± 180	54	-
3a	S166A + Q168K	2	NA	NA	-	-
3a	S166A + Q168L	2	NA	NA	-	-
3a	S166A + Q168R	2	NA	NA	-	-
3a	Y56H + S166A + Q168K	2	NA	NA	-	-
3a	Y56H + S166A + Q168L	2	NA	NA	-	-
3a	Y56H + S166A + Q168R	2	NA	NA	-	-
4a	Wild Type	4	0.67 ± 0.23	2.8 ± 0.66	-	-
4a	P67S	3	0.65 ± 0.27	2.9 ± 0.47	1.0	-
4a	G90R	3	4.7 ± 1.6	7.9 ± 2.6	7.0	-
4a	R155C	3	1.7 ± 0.47	9.2 ± 1.5	2.6	-
4a	A156T	3	962 ± 374	1511 ± 603	1436	-
4a	A156V	3	2081 ± 817	6561 ± 3150	3106	-
4a	D168H	3	14.6 ± 6.1	29.5 ± 2.9	22	-
4a	D168V	3	6.5 ± 3.0	24.5 ± 1.4	9.7	-
4a	P67S + A156T	3	1187 ± 101	3486 ± 736	1772	-
4a	G90R + A156T	3	1343 ± 175	3238 ± 506	2004	-
4d	Wild Type	3	0.15 ± 0.04	0.88 ± 0.08	-	-
4d	Y56H	3	0.69 ± 0.14	5.8 ± 2.9	4.6	-
4d	D168V	3	0.28 ± 0.12	1.6 ± 0.16	1.9	-
4d	Y56H + D168V	3	8.7 ± 0.56	26.3 ± 3.8	58	-
6a	Wild Type	3	0.15 ± 0.03	1.0 ± 0.57	-	-
6a	D168A	3	12.2 ± 5.8	32.0 ± 1.8	81	-
6a	D168G	3	28.6 ± 6.5	59.2 ± 22.1	191	-
6a	D168H	3	22.0 ± 5.7	77.7 ± 17.0	146	-
6a	D168V	3	5.8 ± 2.2	24.3 ± 0.82	38	-
6a	D168Y	3	16.3 ± 4.0	34.2 ± 4.6	109	-
6a	M179T	3	0.19 ± 0.08	1.1 ± 0.47	1.3	-
6a	D168H + M179T	3	22.9 ± 7.2	75.1 ± 14.5	153	-
6a	D168V + M179T	3	8.2 ± 2.9	24.9 ± 2.3	54	-
6a	D168Y + M179T	3	14.4 ± 3.6	28.9 ± 3.9	96	-

Appendix B: Site-directed mutant replicon phenotype data for pibrentasvir (PIB)

Replication capacity (RC) is not shown for chimeric replicons. Highlight indicates EC50 fold-changes (FC) ≥10 relative to subtype parent replicons (arbitrary cutoff). NA, data not available due to low replication capacity.

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC50	RC (%)
1a	Wild-Type (H77)	11	0.72 ± 0.45	1.8 ± 0.65	-	100
1a	K24R	4	0.25 ± 0.04	0.72 ± 0.03	0.3	172
1a	M28A	3	1.4 ± 0.34	4.7 ± 1.8	2.0	31
1a	M28G	3	176 ± 21.4	704 ± 281	244	55
1a	M28T	3	1.5 ± 1.1	4.1 ± 3.4	2.0	100
1a	M28V	3	1.3 ± 0.86	3.7 ± 2.1	1.8	87
1a	Q30D	4	67.7 ± 37.0	244 ± 79.6	94	50
1a	Q30E	3	1.7 ± 0.39	5.1 ± 0.71	2.4	70
1a	Q30G	3	0.93 ± 0.24	3.5 ± 1.2	1.3	47
1a	Q30H	3	0.74 ± 0.21	2.4 ± 1.2	1.0	64
1a	Q30K	6	0.69 ± 0.32	2.9 ± 0.91	1.0	36
1a	Q30L	3	0.21 ± 0.05	0.67 ± 0.07	0.3	52
1a	Q30R	3	1.2 ± 0.62	3.9 ± 2.1	1.7	60
1a	Q30Y	3	0.55 ± 0.11	3.0 ± 0.80	0.8	21
1a	L31M	3	0.76 ± 0.11	1.6 ± 0.34	1.1	141
1a	L31V	3	0.96 ± 0.85	2.4 ± 1.4	1.3	241
1a	P32L	3	1.2 ± 0.43	5.1 ± 0.79	1.7	19
1a	F36L	3	0.64 ± 0.11	2.1 ± 0.62	0.9	85
1a	H58C	3	0.68 ± 0.14	2.5 ± 0.90	0.9	111
1a	H58D	11	0.80 ± 0.17	3.9 ± 0.99	1.1	66
1a	H58P	4	0.46 ± 0.06	1.5 ± 0.20	0.6	129
1a	H58R	3	0.50 ± 0.09	1.7 ± 0.09	0.7	124
1a	E62A	3	3.7 ± 0.15	12.2 ± 1.7	5.2	357
1a	E62D	3	0.46 ± 0.06	1.6 ± 0.14	0.6	104
1a	A92T	3	0.28 ± 0.03	1.6 ± 0.22	0.4	4.1
1a	Y93C	4	1.2 ± 0.57	3.5 ± 2.1	1.7	24
1a	Y93F	3	0.68 ± 0.20	3.0 ± 0.99	0.9	90
1a	Y93H	6	4.8 ± 1.5	22.9 ± 5.6	6.7	18
1a	Y93L	4	0.84 ± 0.10	4.7 ± 0.20	1.2	42
1a	Y93N	4	5.1 ± 2.1	19.6 ± 4.6	7.0	25
1a	Y93S	4	1.2 ± 0.20	8.6 ± 1.1	1.6	3.4
1a	K24R + M28V	4	0.42 ± 0.05	1.4 ± 0.16	0.6	155
1a	K24R + M28T	4	0.41 ± 0.04	1.5 ± 0.23	0.6	107
1a	K24R + Q30R	4	0.29 ± 0.06	1.3 ± 0.12	0.4	83
1a	M28G + Q30R	3	15713 ± 5580	113030 ± 34567	21824	66
1a	M28V + Q30H	3	0.33 ± 0.00	1.2 ± 0.01	0.5	71
1a	M28V + Q30R	4	0.82 ± 0.04	5.9 ± 0.34	1.1	17
1a	M28V + Y93L	4	0.57 ± 0.03	2.9 ± 0.34	0.8	31
1a	M28T + Q30R	3	1.2 ± 0.21	5.2 ± 1.3	1.6	32
1a	M28T + Y93C	3	2.2 ± 0.47	9.0 ± 2.5	3.1	22
1a	Q30H + Y93H	3	12.3 ± 1.2	59.8 ± 6.2	17	35
1a	Q30K + H58D	6	170 ± 40.7	1249 ± 283	235	166
1a	Q30K + Y93H	3	1286 ± 353	7584 ± 1641	1786	14
1a	Q30L + Y93H	3	0.42 ± 0.09	1.8 ± 0.44	0.6	30
1a	Q30L + Y93S	3	NA	NA	-	1.1
1a	Q30R + L31M	6	2.1 ± 0.79	7.7 ± 2.8	3.0	49
1a	Q30R + H58D	6	91.0 ± 37.0	604 ± 280	126	50
1a	Q30R + Y93C	3	2.8 ± 0.64	11.1 ± 1.4	3.8	6.2
1a	Q30R + Y93F	3	4.0 ± 0.94	20.3 ± 2.3	5.5	30
1a	Q30R + Y93H	3	187 ± 110	661 ± 331	260	21
1a	Q30R + Y93N	3	94.6 ± 15.5	647 ± 120	131	3.6
1a	Q30R + Y93S	4	NA	NA	-	< 0.5
1a	Q30Y + F36L	3	0.58 ± 0.13	3.3 ± 1.5	0.8	28
1a	L31M + H58D	3	16.6 ± 2.7	109 ± 46.9	23	214
1a	L31M + Y93C	3	4.4 ± 0.55	19.0 ± 3.6	6.1	32
1a	L31M + Y93H	3	54.3 ± 9.3	295 ± 42.0	75	11

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC50	RC (%)
1a	L31M + Y93N	3	140 ± 34.2	748 ± 202	195	31
1a	L31V + Y93H	3	67.8 ± 36.1	328 ± 97.5	94	20
1a	P32L + Y93C	4	124 ± 27.8	755 ± 144	172	0.5
1a	H58D + Y93C	4	168 ± 32.3	2392 ± 399	233	13
1a	H58D + Y93H	4	1612 ± 272	7764 ± 2077	2238	13
1a	H58D + Y93N	3	1418 ± 279	7773 ± 1700	1969	21
1a	H58D + Y93S	4	1058 ± 457	8701 ± 2752	1469	2.1
1a	A92T + Y93N	3	NA	NA	-	< 0.5
1a	M28G + Q30R + H58C	3	679 ± 115	7268 ± 3891	942	63
1a	M28T + Q30R + L31M	3	3.3 ± 0.41	14.2 ± 0.48	4.6	45
1a	Q30H + H58D + Y93H	3	6737 ± 1085	30913 ± 8712	9357	16
1a	Q30K + H58D + E62A	3	99.5 ± 3.9	543 ± 30.9	138	90
1a	Q30R + L31M + H58D	3	1227 ± 277	7298 ± 2203	1704	77
1a	Q30R + L31M + Y93C	3	30.0 ± 1.0	136 ± 5.9	42	7.1
1a	Q30Y + F36L + Y93H	3	19.8 ± 2.6	88.1 ± 23.1	28	8.6
1b	Wild-Type (Con1)	12	1.9 ± 0.80	3.8 ± 1.2	-	100
1b	L28M	3	1.8 ± 0.11	3.5 ± 0.42	1.0	114
1b	L28T	4	1.7 ± 0.44	3.8 ± 1.1	0.9	17
1b	P29 deletion	3	19.3 ± 6.8	85.7 ± 17.4	10	5.6
1b	R30Q	3	0.88 ± 0.49	2.2 ± 1.3	0.5	ND
1b	L31F	3	2.3 ± 0.37	4.5 ± 0.91	1.2	127
1b	L31M	3	2.9 ± 1.2	5.8 ± 2.2	1.5	119
1b	L31V	3	1.5 ± 0.43	2.8 ± 0.62	0.8	86
1b	P32 deletion	?	1968 ± 203	?	1036	?
1b	Q54H	3	2.1 ± 0.27	5.3 ± 0.98	1.1	120
1b	Q54Y	3	2.3 ± 0.89	7.1 ± 0.47	1.2	112
1b	P58S	3	2.4 ± 1.3	4.7 ± 2.0	1.2	80
1b	A92E	3	0.92 ± 0.23	2.7 ± 0.25	0.5	29
1b	A92V	3	0.86 ± 0.15	2.1 ± 0.23	0.5	54
1b	Y93H	10	1.1 ± 0.27	3.9 ± 1.2	0.6	73
1b	Y93N	3	1.2 ± 0.25	3.7 ± 0.84	0.6	52
1b	Y93S	3	0.74 ± 0.24	3.0 ± 0.29	0.4	23
1b	L28M + R30Q	3	0.79 ± 0.10	1.9 ± 0.24	0.4	28
1b	L28M + P32 deletion	?	12020 ± 2148	?	6326	?
1b	L28M + Y93H	3	2.2 ± 0.23	5.1 ± 0.17	1.2	104
1b	R30Q + Y93H	3	2.3 ± 0.15	4.8 ± 0.40	1.2	60
1b	L31F + Q54H	3	4.5 ± 0.87	9.2 ± 1.8	2.4	99
1b	L31F + A92E	3	1.2 ± 0.15	3.5 ± 0.49	0.6	36
1b	L31F + Y93H	3	2.8 ± 0.17	8.4 ± 2.1	1.5	35
1b	L31M + Y93H	3	1.3 ± 0.24	4.3 ± 0.41	0.7	11
1b	L31V + Y93H	3	1.7 ± 0.31	4.5 ± 0.38	0.9	24
1b	Q54H + A92E	3	1.7 ± 0.28	4.3 ± 0.31	0.9	62
1b	Q54H + Y93H	3	1.7 ± 0.31	4.5 ± 0.68	0.9	35
1b	Q54Y + Y93H	3	2.0 ± 0.33	7.0 ± 0.56	1.0	34
1b	P58S + Y93H	3	1.5 ± 0.45	5.7 ± 2.5	0.8	34
1b	A92V + Y93H	3	0.68 ± 0.29	2.6 ± 0.98	0.4	2.5
1b	L28M + R30Q + Y93H	3	1.0 ± 0.24	3.2 ± 0.29	0.5	28
1b	L31F + Q54H + A92E	3	3.2 ± 0.57	8.2 ± 1.3	1.7	70
1b	Q54H + A92V + Y93H	3	1.8 ± 0.70	5.2 ± 0.68	1.0	1.4
2a	Wild Type (JFH-1)	14	0.99 ± 0.36	4.4 ± 1.6	-	-
2a	T24A	5	1.3 ± 0.30	4.5 ± 1.9	1.3	-
2a	T24S	3	1.0 ± 0.55	4.8 ± 2.2	1.1	-
2a	F28C	3	1.3 ± 0.21	4.5 ± 0.40	1.3	-
2a	F28S	3	1.2 ± 0.17	7.4 ± 0.75	1.2	-
2a	P29S	3	NA	NA	-	-
2a	K30G	3	0.75 ± 0.11	3.3 ± 0.43	0.8	-
2a	K30M	3	1.2 ± 0.16	4.6 ± 0.08	1.2	-
2a	M31V	3	NA	NA	-	-
2a	M31I	6	1.2 ± 0.22	5.6 ± 2.9	1.2	-
2a	C92S	3	1.6 ± 0.10	10.3 ± 2.9	1.6	-
2a	Y93H	3	NA	NA	-	-
2a	T24A + M31L	3	0.80 ± 0.30	5.8 ± 2.3	0.8	-
2a	T24A + C92S	3	1.7 ± 0.23	5.1 ± 1.1	1.7	-

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC50	RC (%)
2a	T24S + F28C	3	1.4 ± 0.17	6.0 ± 2.8	1.4	-
2a	F28S + M31I	3	14303 ± 2722	39977 ± 10415	14448	-
2a	F28S + Y93H	3	NA	NA	-	-
2a	P29S + K30G	4	2.3 ± 0.36	12.9 ± 1.7	2.3	-
2b	Wild Type	24	1.2 ± 0.39	4.8 ± 2.4	-	-
2b	L27M	3	0.92 ± 0.41	15.2 ± 8.8	0.8	-
2b	L28F	10	0.94 ± 0.27	4.7 ± 1.6	0.8	-
2b	L31I	3	1.8 ± 0.15	4.9 ± 0.74	1.5	-
2b	L31M	3	1.5 ± 0.33	5.7 ± 2.3	1.2	-
2b	L31V	8	0.64 ± 0.20	2.8 ± 0.72	0.5	-
2b	V52I	3	1.2 ± 0.37	4.5 ± 0.11	1.0	-
2b	C92S	12	1.1 ± 0.28	4.4 ± 1.6	0.9	-
2b	C92Y	6	0.69 ± 0.19	4.6 ± 1.5	0.6	-
2b	Y93H	4	NA	NA	-	-
2b	Y93N	3	NA	NA	-	-
2b	L28F + L31I	3	1.3 ± 0.29	6.1 ± 2.4	1.1	-
2b	L28F + L31M	5	1.4 ± 0.27	8.4 ± 4.0	1.2	-
2b	L28F + L31V	3	NA	NA	-	-
2b	L31M + C92S	3	0.96 ± 0.19	5.8 ± 1.7	0.8	-
2b	L31M + C92Y	3	0.80 ± 0.15	4.6 ± 1.3	0.7	-
2b	L31V + C92S	3	0.59 ± 0.03	2.9 ± 0.28	0.5	-
2b	L28F + L31M + C92S	3	2.0 ± 0.54	14.3 ± 4.1	1.6	-
2b	L28F + L31M + C92Y	3	NA	NA	-	-
3a	Wild Type	16	0.65 ± 0.16	1.7 ± 0.27	-	-
3a	S24F	3	NA	NA	-	-
3a	M28G	3	NA	NA	-	-
3a	M28K	3	NA	NA	-	-
3a	M28T	3	1.1 ± 0.02	2.1 ± 0.13	1.7	-
3a	A30K	6	0.71 ± 0.18	2.8 ± 0.44	1.1	-
3a	L31F	4	NA	NA	-	-
3a	L31I	3	NA	NA	-	-
3a	Y93H	4	1.5 ± 0.19	9.5 ± 2.8	2.3	-
3a	S24F + M28K	3	173 ± 65.1	865 ± 82.2	267	-
3a	S24F + A30K	3	2.1 ± 0.14	4.4 ± 0.03	3.3	-
3a	M28K + A30K	3	NA	NA	-	-
3a	A30K + L31I	3	2.4 ± 0.03	11.6 ± 2.3	3.6	-
3a	A30K + Y93H	3	45.1 ± 2.3	180 ± 17.4	69	-
3a	L31F + Y93H	3	NA	NA	-	-
3a	L31I + Y93H	3	9.6 ± 1.5	58.3 ± 13.3	15	-
3a	S24F + M28K + A30K	3	8932 ± 1517	38547 ± 3825	13742	-
3a	A30K + L31I + Y93H	3	2770 ± 353	9134 ± 1118	4262	-
4a	Wild Type	14	0.78 ± 0.14	2.0 ± 0.46	-	-
4a	L28I	3	0.80 ± 0.07	2.3 ± 0.38	1.0	-
4a	L28M	3	0.63 ± 0.17	1.8 ± 0.18	0.8	-
4a	L28V	3	0.85 ± 0.23	2.7 ± 1.5	1.1	-
4a	L30H	3	1.1 ± 0.51	2.6 ± 0.52	1.3	-
4a	P58L	3	0.75 ± 0.07	2.5 ± 0.81	1.0	-
4a	Y93H	3	NA	NA	-	-
4a	L28I + P58L	3	1.3 ± 0.11	3.5 ± 1.1	1.7	-
4a	L28M + P58L	3	NA	NA	-	-
4a	P58L + Y93H	3	NA	NA	-	-
4d	Wild Type	15	1.5 ± 0.55	4.1 ± 1.2	-	-
4d	L28S	3	NA	NA	-	-
4d	L28V	3	1.7 ± 0.29	8.2 ± 2.2	1.1	-
4d	M31I	3	2.1 ± 0.20	4.3 ± 0.56	1.4	-
4d	M31L	3	1.4 ± 0.28	4.6 ± 0.60	1.0	-
4d	T58A	3	1.8 ± 0.32	6.3 ± 0.95	1.2	-
4d	T58P	3	1.7 ± 0.31	6.7 ± 1.1	1.1	-
4d	T58S	3	1.5 ± 0.16	4.1 ± 0.32	1.0	-
4d	Y93H	4	NA	NA	-	-
4d	K24Q + T58P	4	2.0 ± 0.20	4.0 ± 1.0	1.3	-
4d	L28S + M31I	3	NA	NA	-	-
4d	L28V + T58S	3	1.7 ± 0.26	6.9 ± 0.87	1.1	-

Subtype	NS5A Amino Acid Substitutions	N	Mean EC_{50} ± SD (pM)	Mean EC_{90} ± SD (pM)	FC EC50	RC (%)
4d	T58P + Y93H	4	NA	NA	-	-
4d	K24Q + T58P + Y93H	4	1.5 ± 0.27	6.3 ± 1.5	1.0	-
5a	Wild Type	4	0.93 ± 0.20	4.3 ± 0.97	-	-
5a	L28I	3	0.98 ± 0.14	6.2 ± 2.3	1.1	-
5a	L31F	3	1.9 ± 0.11	10.5 ± 3.7	2.1	-
5a	L31V	3	0.75 ± 0.24	3.3 ± 0.69	0.8	-
6a	Wild Type	4	1.0 ± 0.31	3.3 ± 1.0	-	-
6a	L31V	3	1.0 ± 0.38	3.1 ± 0.67	1.0	-
6a	T58A	3	1.4 ± 0.45	4.5 ± 1.4	1.4	-
6a	T58N	3	1.8 ± 0.71	9.6 ± 3.4	1.8	-

Appendix C: Listing of SVR12 and virologic failure results from individual trials of NS3/4A PI-naïve/NS5A inhibitor-naïve subjects, GLE/PIB to-be-marketed dose levels.

					SVR-FDA		VF Category			
HCV GT	CIRR	Tx History	STUDYID	Regimen	N	Y	Any VF	On-Tx VF	Relapse	Non-VF
1	N	Naïve	M13-590	GLE/PIB 8W	2	217	0	0	0	2
				GLE/PIB 12W	1	216	0	0	0	1
			M14-867	GLE/PIB 8W	1	28	0	0	0	1
			M14-868	GLE/PIB 12W	0	1	0	0	0	0
			M15-462	GLE/PIB 12W	0	23	0	0	0	0
			M15-464	GLE/PIB 12W	0	1	0	0	0	0
		IFN- or P/R-experienced	M13-590	GLE/PIB 8W	1	130	1	1	0	0
				GLE/PIB 12W	0	133	0	0	0	0
			M14-867	GLE/PIB 8W	0	5	0	0	0	0
			M14-868	GLE/PIB 8W	0	2	0	0	0	0
			M15-462	GLE/PIB 12W	0	20	0	0	0	0
			M15-464	GLE/PIB 12W	0	2	0	0	0	0
		SOF + RBV or P/R	M13-590	GLE/PIB 8W	0	1	0	0	0	0
				GLE/PIB 12W	0	2	0	0	0	0
			M15-462	GLE/PIB 12W	0	1	0	0	0	0
			M15-464	GLE/PIB 12W	0	1	0	0	0	0
	Y	Naïve	M14-172	GLE/PIB 12W	0	66	0	0	0	0
			M15-462	GLE/PIB 12W	2	3	0	0	0	2
		IFN- or P/R-experienced	M14-172	GLE/PIB 12W	1	19	1	0	1	0
			M15-462	GLE/PIB 12W	0	5	0	0	0	0
		SOF + RBV or P/R	M14-172	GLE/PIB 12W	0	4	0	0	0	0
			M15-462	GLE/PIB 12W	0	1	0	0	0	0
2	N	Naïve	M14-868	GLE/PIB 8W	2	172	0	0	0	2
				GLE/PIB 12W	1	20	0	0	0	1
			M15-462	GLE/PIB 12W	0	8	0	0	0	0
			M15-464	GLE/PIB 12W	1	139	0	0	0	1
		IFN- or P/R-experienced	M14-868	GLE/PIB 8W	1	16	1	0	1	0
				GLE/PIB 12W	0	3	0	0	0	0
			M15-462	GLE/PIB 12W	0	4	0	0	0	0
			M15-464	GLE/PIB 12W	0	53	0	0	0	0
		SOF + RBV or P/R	M14-868	GLE/PIB 8W	1	5	1	0	1	0
			M15-464	GLE/PIB 12W	0	5	0	0	0	0
	Y	Naïve	M14-172	GLE/PIB 12W	0	24	0	0	0	0
			M15-462	GLE/PIB 12W	0	2	0	0	0	0
		IFN- or P/R-experienced	M14-172	GLE/PIB 12W	0	1	0	0	0	0
			M15-462	GLE/PIB 12W	0	2	0	0	0	0
		SOF + RBV or P/R	M14-172	GLE/PIB 12W	0	6	0	0	0	0
3	N	Naïve	M13-594	GLE/PIB 8W	8	149	6	1	5	2
				GLE/PIB 12W	11	222	4	1	3	7
				SOF+DCV 12W	4	111	1	0	1	3
			M14868	GLE/PIB 8W	1	28	0	0	0	1
				GLE/PIB 12W	1	26	0	0	0	1
			M15-462	GLE/PIB 12W	0	10	0	0	0	0
		IFN- or P/R-experienced	M14868	GLE/PIB 12W	5	36	5	1	4	0
				GLE/PIB 16W	1	12	1	0	1	0
		SOF + RBV or P/R	M14868	GLE/PIB 12W	0	7	0	0	0	0
				GLE/PIB 16W	0	9	0	0	0	0
		SOF/LDV	M14868	GLE/PIB 12W	0	1	0	0	0	0
	Y	Naïve	M14868	GLE/PIB 12W	1	63	0	0	0	1
				GLE/PIB + RBV 12W	0	24	0	0	0	0
			M15-462	GLE/PIB 12W	0	1	0	0	0	0
		IFN- or P/R-experienced	M14868	GLE/PIB 16W	2	24	2	1	1	0
				GLE/PIB + RBV 12W	0	3	0	0	0	0
		SOF + RBV or P/R	M14868	GLE/PIB 16W	1	24	1	0	1	0

					SVR-FDA		VF Category			
HCV GT	**CIRR**	**Tx History**	**STUDYID**	**Regimen**	**N**	**Y**	**Any VF**	**On-Tx VF**	**Relapse**	**Non-VF**
4	N	Naïve	M13-583	GLE/PIB 12W	0	45	0	0	0	0
			M14-867	GLE/PIB 12W	0	16	0	0	0	0
			M14-868	GLE/PIB 8W	3	36	0	0	0	3
			M15-462	GLE/PIB 12W	0	10	0	0	0	0
		IFN- or P/R-experienced	M13-583	GLE/PIB 12W	1	30	0	0	0	1
			M14-867	GLE/PIB 12W	0	4	0	0	0	0
			M14-868	GLE/PIB 8W	0	7	0	0	0	0
			M15-462	GLE/PIB 12W	0	6	0	0	0	0
	Y	Naïve	M14-172	GLE/PIB 12W	0	12	0	0	0	0
		IFN- or P/R-experienced	M14-172	GLE/PIB 12W	0	3	0	0	0	0
			M15-462	GLE/PIB 12W	0	4	0	0	0	0
		SOF + RBV or P/R	M14-172	GLE/PIB 12W	0	1	0	0	0	0
5	N	Naïve	M13-583	GLE/PIB 12W	0	20	0	0	0	0
			M14-867	GLE/PIB 12W	0	1	0	0	0	0
			M14-868	GLE/PIB 8W	0	2	0	0	0	0
			M15-462	GLE/PIB 12W	0	1	0	0	0	0
		IFN- or P/R-experienced	M13-583	GLE/PIB 12W	0	6	0	0	0	0
	Y	Naïve	M14-172	GLE/PIB 12W	0	2	0	0	0	0
6	N	Naïve	M13-583	GLE/PIB 12W	0	17	0	0	0	0
			M14-867	GLE/PIB 12W	0	10	0	0	0	0
			M14-868	GLE/PIB 8W	1	7	0	0	0	1
		IFN- or P/R-experienced	M13-583	GLE/PIB 12W	0	2	0	0	0	0
			M14-867	GLE/PIB 12W	0	1	0	0	0	0
			M14-868	GLE/PIB 8W	0	2	0	0	0	0
			M15-462	GLE/PIB 12W	0	1	0	0	0	0
	Y	Naïve	M14-172	GLE/PIB 12W	0	6	0	0	0	0
		IFN- or P/R-experienced	M14-172	GLE/PIB 12W	0	1	0	0	0	0

Appendix D: HCV subtype-specific reference strains for NS3/4A and NS5A sequence analyses, and reference amino acid sequences at positions of interest.

SUBTYPE	ACCESSNO	STRAIN	NS30036	NS30043	NS30054	NS30055	NS30056	NS30080	NS30107	NS30122	NS30132	NS30155	NS30156	NS30158	NS30166	NS30168	NS30170
1A	NC_004102	H77	V	F	T	V	Y	Q	V	S	I	R	A	V	A	D	I
1B	AJ238799	Con1	V	F	T	V	Y	Q	V	S	V	R	A	V	A	D	V
1C	D14853	HC-G9	V	F	T	V	Y	Q	V	N	I	R	A	V	A	D	V
1D	KJ439768	QC103	V	F	S	V	Y	K	I	S	I	R	A	V	A	D	V
1E	KJ439769	QC172	L	F	S	V	Y	Q	V	T	V	R	A	V	A	D	V
1G	AM910652	1804	V	F	S	V	Y	Q	V	S	V	R	A	V	A	D	V
1H	KC248198	EBW443	V	F	T	V	Y	Q	V	S	V	R	A	V	A	D	V
1I	KJ439772	QC181	V	F	T	V	Y	Q	V	S	I	R	A	V	A	D	V
1J	KJ439773	QC329	V	F	T	V	Y	Q	V	N	I	R	A	V	A	D	V
1K	KJ439774	QC82	V	F	T	V	Y	Q	V	T	I	R	A	V	A	D	I
1L	KC248193	136142	V	F	T	V	Y	L	V	S	L	R	A	V	A	D	V
1M	KJ439778	QC196	V	F	T	V	Y	Q	V	S	I	R	A	V	A	D	I
1N	KJ439775	QC113	V	F	T	V	F	L	V	S	I	R	A	V	A	D	I
2A	AB047639	JFH-1	L	F	T	V	Y	G	V	K	I	R	A	V	S	D	I
2B	D10988	HC-J8	L	F	T	V	Y	G	V	R	L	R	A	V	S	D	I
2C	D50409	BEBE1	L	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2D	JF735114	QC259	L	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2E	JF735120	QC64	L	F	A	V	F	G	V	R	L	R	A	V	S	D	I
2F	KC844042	ZS542	L	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2I	DQ155561	D54	L	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2J	HM777359	C1292	M	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2K	AB031663	VAT96	L	F	T	V	F	G	V	R	L	R	A	I	S	D	I
2L	KC197235	MRS89	L	F	T	V	Y	G	V	R	L	R	A	V	A	D	V
2M	JF735111	QC178	L	F	T	V	F	G	V	R	I	R	A	V	S	D	I
2Q	FN666429	852	L	F	T	V	F	G	V	R	L	R	A	V	S	D	I
2R	JF735115	QC283	L	F	T	V	F	G	V	T	L	R	A	V	S	D	I
2T	KC197238	MRS40	L	F	T	V	F	G	V	R	L	R	A	V	A	D	I
3A	GU814263	S52	L	F	T	V	Y	Q	V	S	L	R	A	V	A	Q	V
3B	D49374	HCV-Tr	L	F	T	V	Y	Q	V	S	L	R	A	V	A	Q	I
3D	KJ470619	NE274	L	F	T	V	Y	Q	V	S	L	R	A	V	S	Q	I
3E	KJ470618	NE145	L	F	T	V	Y	Q	I	S	L	R	A	V	G	Q	I
3G	JX227954	BID-G1243	L	F	T	V	Y	Q	V	S	L	R	A	V	S	Q	I
3H	JF735121	QC29	L	F	T	V	Y	Q	V	S	L	R	A	I	S	Q	I
3I	FJ407092	IND-HCV-3i	L	F	T	V	Y	Q	V	S	I	R	A	V	A	Q	V
3K	D63821	JK049	L	F	T	V	Y	Q	I	S	L	R	A	V	A	Q	I
4A	GU814265	ED43	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4B	FJ462435	QC264	L	F	T	V	Y	Q	V	S	I	R	A	V	A	D	V
4C	FJ462436	QC381	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4D	FJ462437	QC382	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4F	EF589161	IFBT88	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4G	FJ462432	QC193	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4K	FJ462438	QC383	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4L	FJ839870	QC274	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4M	FJ462433	QC249	L	F	T	V	Y	Q	V	S	I	R	A	V	A	D	I
4N	FJ462441	QC97	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4O	FJ462440	QC93	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4P	FJ462431	QC139	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4Q	FJ462434	QC262	L	F	T	V	Y	Q	V	N	L	R	A	V	A	D	V
4R	FJ462439	QC384	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4T	FJ839869	QC155	L	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
4V	HQ537008	CYHCV048	L	F	T	V	Y	Q	V	S	I	R	A	V	A	D	V
5A	AF064490	SA13	L	F	T	V	F	K	V	T	I	R	A	V	A	D	I
6A	Y12083	EUHK2	V	F	T	V	Y	L	V	S	I	R	A	V	S	D	I
6B	NC_009827	Th580	V	F	T	V	Y	Q	V	T	I	R	A	V	A	D	I
6C	EF424629	Th846	V	F	T	V	Y	Q	V	S	L	R	A	V	A	D	V
6D	D84263	VN235	L	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6E	DQ314805	GX004	V	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6F	DQ835760	C-0044	L	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6G	DQ314806	HK6554	L	F	T	V	Y	Q	I	N	I	R	A	V	A	E	V
6H	D84265	VN004	V	F	T	V	Y	Q	V	N	I	R	A	V	S	D	A
6I	DQ835762	C-0159	V	F	T	V	Y	Q	V	S	I	R	A	V	S	D	V
6J	DQ835769	Th553	V	F	T	V	Y	Q	V	N	I	R	A	V	S	D	V
6K	DQ278893	KM41	V	F	T	V	Y	Q	V	T	I	R	A	V	S	D	V
6L	EF424628	537796	V	F	T	V	Y	Q	I	S	I	R	A	V	S	D	V
6M	DQ835765	C-0185	V	F	T	V	Y	Q	I	T	I	R	A	V	S	D	I
6N	AY878652	KM42	V	F	T	V	Y	Q	V	N	I	R	A	V	S	D	V
6O	EF424627	QC227	I	F	T	V	Y	Q	V	T	I	R	A	V	A	D	V
6P	EF424626	QC216	V	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6Q	EF424625	QC99	I	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6R	EU408328	QC245	V	F	T	V	F	Q	V	T	L	R	A	V	A	D	V
6S	EU408329	QC66	L	F	T	V	F	Q	V	T	L	R	A	V	A	D	V
6T	EU246939	D49	L	F	T	V	F	Q	V	T	L	R	A	V	A	D	V
6U	EU246940	D83	V	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6V	EU798761	KM046	V	F	T	V	Y	Q	V	T	L	R	A	V	A	D	V
6W	EU643836	HCV-6-D370	L	F	T	V	Y	Q	V	S	I	R	A	V	A	D	V
6XA	EU408330	DH012	V	F	T	V	F	Q	V	T	L	R	A	V	A	D	V
6XB	JX183552	TV476	V	F	T	V	Y	Q	V	T	I	R	A	V	S	D	I
6XC	KJ567651	6_TV520	V	F	T	V	Y	Q	V	T	L	R	A	V	A	D	I
6XD	KM252791	L394	L	F	T	V	Y	L	V	N	I	R	A	I	A	D	V
6XE	JX183557	DH027	V	F	T	V	Y	Q	I	T	I	R	A	V	S	D	V
7A	EF108306	QC69	L	F	G	P	Y	D	V	G	V	R	A	V	A	Q	V

SUBTYPE	ACCESSNO	STRAIN	NS5A0024	NS5A0028	NS5A0029	NS5A0030	NS5A0031	NS5A0032	NS5A0058	NS5A0092	NS5A0093
1A	NC_004102	H77	K	M	P	Q	L	P	H	A	Y
1B	AJ238799	Con1	Q	L	P	R	L	P	P	A	Y
1C	D14853	HC-G9	K	M	P	Q	L	P	P	A	H
1D	KJ439768	QC103	K	L	P	R	M	P	P	A	Y
1E	KJ439769	QC172	R	M	P	Q	L	P	P	T	Y
1G	AM910652	1804	S	L	P	R	L	P	P	A	F
1H	KC248198	EBW443	Q	L	P	R	L	P	P	A	Y
1I	KJ439772	QC181	Q	L	P	R	L	P	P	A	Y
1J	KJ439773	QC329	K	M	P	Q	L	P	P	A	Y
1K	KJ439774	QC82	K	A	P	Q	L	P	P	A	Y
1L	KC248193	136142	G	M	P	R	M	P	P	A	Y
1M	KJ439778	QC196	K	M	P	Q	M	P	P	A	C
1N	KJ439775	QC113	K	M	P	Q	L	P	P	A	Y
2A	AB047639	JFH-1	T	F	P	K	L	P	P	C	Y
2B	D10988	HC-J8	S	L	P	K	M	P	P	C	Y
2C	D50409	BEBE1	S	F	P	R	L	P	P	C	Y
2D	JF735114	QC259	S	L	P	K	L	P	P	C	Y
2E	JF735120	QC64	S	F	P	K	M	P	S	S	Y
2F	KC844042	ZS542	F	S	P	K	M	P	P	S	Y
2I	DQ155561	D54	S	F	P	K	M	P	P	C	Y
2J	HM777359	C1292	S	L	P	K	M	P	S	C	Y
2K	AB031663	VAT96	S	L	P	K	M	P	P	C	Y
2L	KC197235	MRS89	S	L	P	K	L	P	P	S	Y
2M	JF735111	QC178	S	L	P	R	M	P	P	S	Y
2Q	FN666429	852	S	L	P	K	M	P	P	C	Y
2R	JF735115	QC283	S	F	P	K	M	P	P	C	Y
2T	KC197238	MRS40	S	L	P	K	L	P	P	C	Y
3A	GU814263	S52	S	M	P	A	L	P	P	E	Y
3B	D49374	HCV-Tr	S	M	P	K	V	P	P	E	Y
3D	KJ470619	NE274	S	M	P	K	M	P	P	E	Y
3E	KJ470618	NE145	S	M	P	K	L	P	P	E	Y
3G	JX227954	BID-G1243	S	M	P	K	V	P	P	E	Y
3H	JF735121	QC29	S	M	P	A	L	P	P	E	Y
3I	FJ407092	ND-HCV-3i	S	M	P	K	L	P	P	E	Y
3K	D63821	JK049	G	L	P	K	M	P	P	G	Y
4A	GU814265	ED43	K	L	P	L	M	P	P	A	Y
4B	FJ462435	QC264	K	L	P	S	M	P	P	T	H
4C	FJ462436	QC381	K	L	P	R	M	P	P	A	Y
4D	FJ462437	QC382	K	L	P	R	M	P	T	A	Y
4F	EF589161	FBT88	K	L	P	Q	M	P	P	A	Y
4G	FJ462432	QC193	K	L	P	L	M	P	P	A	H
4K	FJ462438	QC383	K	L	P	R	M	P	P	A	Y
4L	FJ839870	QC274	K	L	P	R	M	P	P	A	Y
4M	FJ462433	QC249	K	L	P	S	M	P	P	A	Y
4N	FJ462441	QC97	K	L	P	R	M	P	T	A	Y
4O	FJ462440	QC93	K	M	P	T	M	P	P	A	Y
4P	FJ462431	QC139	K	L	P	R	M	P	P	A	Y
4Q	FJ462434	QC262	K	L	P	R	M	P	P	A	Y
4R	FJ462439	QC384	K	I	P	R	L	P	P	A	Y
4T	FJ839869	QC155	K	L	P	R	M	P	P	A	Y
4V	HQ537008	CYHCV048	K	L	P	R	M	P	P	A	Y
5A	AF064490	SA13	Q	L	P	Q	L	P	P	A	T
6A	Y12083	EUHK2	Q	F	P	R	L	P	T	A	T
6B	NC_009827	Th580	K	T	P	R	I	P	S	A	T
6C	EF424629	Th846	K	V	P	A	L	P	G	P	T
6D	D84263	VN235	R	V	P	T	L	P	S	A	I
6E	DQ314805	GX004	K	V	P	S	L	P	P	A	T
6F	DQ835760	C-0044	K	A	P	S	L	P	S	A	T
6G	DQ314806	HK6554	K	M	P	N	L	P	P	A	T
6H	D84265	VN004	K	V	P	A	L	P	P	A	T
6I	DQ835762	C-0159	K	V	P	A	L	P	P	A	T
6J	DQ835769	Th553	K	V	P	A	L	P	P	A	T
6K	DQ278893	KM41	K	V	P	A	L	P	P	A	T
6L	EF424628	537796	K	V	P	A	L	P	P	A	T
6M	DQ835765	C-0185	K	V	P	S	L	P	T	A	S
6N	AY878652	KM42	K	V	P	S	L	P	T	A	S
6O	EF424627	QC227	K	L	P	A	L	P	A	A	S
6P	EF424626	QC216	K	V	P	S	L	P	P	A	T
6Q	EF424625	QC99	K	V	P	S	L	P	P	A	T
6R	EU408328	QC245	K	G	P	A	L	P	P	A	T
6S	EU408329	QC66	K	L	P	S	L	P	P	A	T
6T	EU246939	D49	K	V	P	S	L	P	G	A	T
6U	EU246940	D83	K	V	P	S	L	P	P	S	T
6V	EU798761	KM046	K	V	P	S	L	P	P	A	S
6W	EU643836	HCV-6-D370	K	F	P	A	L	P	P	A	T
6XA	EU408330	DH012	K	V	P	S	L	P	P	A	T
6XB	JX183552	TV476	K	V	P	A	L	P	P	A	T
6XC	KJ567651	6_TV520	K	V	P	A	L	P	P	A	T
6XD	KM252791	L394	K	F	P	R	L	P	P	A	T
6XE	JX183557	DH027	K	V	P	S	L	P	T	A	S
7A	EF108306	QC69	K	L	P	S	L	P	P	S	H

Appendix E: Individual subject listing of HCV resistance-associated substitutions detected in subjects who experienced virologic failure in Phase 2b/3 trials of GLE/PIB, NS3/4A PI-naïve/NS5A inhibitor-naïve subjects, GLE/PIB to-be-marketed dose levels.

HCV GT	USUBJID	Cirr?	Tx Hist	Regimen	VF Cat	VISIT	Target	Subst.	AA Freq	Tx-Emerg	Tx-Enrch
1a	M13590-50890-106104	N	IFN- or P/R-exp	GLE/PIB 8W	On-Tx VF	BASELINE	NS5A	Q30R	12.47		
								L31M	13.92		
						WEEK 8	NS3/4A	A156V	99.85	Y	
							NS5A	Q30R	98.45		Y
								L31M	99.58		Y
								H58D	97.37	Y	
								H58Y	2.25	Y	
	M14172-48171-203501	Y	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS5A	K24R	6.23		
								Y93N	46.22		
						PTW 8	NS5A	Q30R	87.79	Y	
								H58D	13.51	Y	
								Y93N	99.73		Y
2a	M14868-22887-2917	N	SOF-exp (+/- IFN or RBV)	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	I132L	99.55		
							NS5A	L31M	99.44		
						PTW 4	NS3/4A	I132L	99.84		
							NS5A	L31M	99.93		
	M14868-32999-7016	N	IFN- or P/R-exp	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	I132L	99.34		
							NS5A	L31M	99.69		
						PTW 8	NS3/4A	I132L	99.67		
							NS5A	L31M	99.87		
3a	M13594-07584-174309	N	Naïve	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	V170I	99.75		
							NS5A	A30K	99.73		
						PTW 4	NS3/4A	Q168L	98.91	Y	
								V170I	99.85		
							NS5A	A30K	99.83		
								Y93H	99.7	Y	
	M13594-09818-162318	N	Naïve	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	V170I	99.9		
							NS5A	A30K	99.84		
						PTW 2	NS3/4A	Y56H	99.51	Y	
								V170I	99.92		
							NS5A	A30K	99.79		
								Y93H	99.56	Y	
	M13594-15494-123305	N	Naïve	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	A166S	52.36		
								V170I	99.83		
							NS5A	A30K	99.81		
						PTW 4	NS3/4A	Y56H	88.48	Y	
								A166S	3.84		
								Q168L	95.51	Y	
								V170I	99.88		
							NS5A	A30K	99.81		
								Y93H	99.75	Y	
	M13594-32999-129306	N	Naïve	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	T54S	98.38		
								V170I	96.28		
						PTW 4	NS3/4A	T54S	99.36		
								V170I	98.96		
	M13594-45207-137304	N	Naïve	GLE/PIB 8W	On-Tx VF	BASELINE	NS3/4A	A166S	60.61		
								Q168R	61.44		
								V170I	99.77		
							NS5A	A30K	99.58		
						WEEK 8	NS3/4A	Q80R	59.24	Y	
								A156G	99.58	Y	
								V170I	99.94		
							NS5A	A30K	99.9		
								Y93H	99.71	Y	
	M13594-45400-139305	N	Naïve	GLE/PIB 12W	On-Tx VF	BASELINE	NS3/4A	Q168R	28.5		
								V170I	99.77		

							NS5A	A30K	16.32		
								A30V	39.22		
								Y93H	37.19		
						WEEK 12	NS3/4A	Y56H	98.51	Y	
								Q168R	99.22		Y
								V170I	99.85		
							NS5A	A30K	99.86		Y
								E92*	2	Y	
								Y93H	99.82		Y
	M13594-47653-141305	N	Naïve	GLE/PIB 12W	Relapse	BASELINE	NS3/4A	L132I	4.06		
								V170I	99.82		
						PTW 4	NS3/4A	V170I	99.8		
							NS5A	A30G	32.8	Y	
								Y93H	99.87	Y	
	M13594-53762-140310	N	Naïve	GLE/PIB 8W	Relapse	BASELINE	NS3/4A	A166S	99.16		
								V170I	99.91		
						PTW 12	NS3/4A	A166S	97.7		
								A166Y	2.26	Y	
								V170I	99.93		
							NS5A	E92*	3.37	Y	
								Y93H	99.57	Y	
	M13594-82703-128308	N	Naïve	SOF+DCV 12W	Relapse	BASELINE	NS5A	Y93H	82.62		
						PTW 4	NS5A	Y93H	99.65		
	M14868-12641-1301	N	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS3/4A	V170I	99.86		
							NS5A	P29Q	2.03		
								A30K	99.76		
								E92*	2.57		
						PTW 2	NS3/4A	Y56H	90.79	Y	
								Y56N	3.93	Y	
								Q80K	2.16	Y	
								Q80R	12.59	Y	
								Q168R	83.55	Y	
								V170I	99.97		
							NS5A	P29Q	2.5		
								A30K	99.93		
								P58T	2.16	Y	
								E92*	3.75		
								Y93H	99.54	Y	
						PTW 24	NS3/4A	Q80R	12.06	Y	
								V170I	99.9		
							NS5A	A30K	99.76		
								Y93H	7.31	Y	
	M14868-32999-7003	N	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS3/4A	L132I	99.89		
								V170I	99.8		
							NS5A	A30V	3.71		
								E92*	2.03		
								Y93H	38.67		
						PTW 24	NS5A	Y93H	99.79		Y
						PTW 8	NS3/4A	Q80K	2.65	Y	
								L132I	99.9		
								V170I	99.87		
							NS5A	L31F	38.86	Y	
								E92*	3.92		
								Y93H	99.29		Y
	M14868-40525-3409	N	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS3/4A	Q80K	2.48		
								L132I	99.88		
								V170I	99.86		
							NS5A	A30V	4.61		
								P58T	2.61		
								E92*	4.2		
								Y93H	99.31		
						PTW 4	NS3/4A	L132I	99.89		
								V170I	99.94		

							NS5A	Y93H	99.58		
	M14868-40537-6119	Y	SOF-exp (+/- IFN or RBV)	GLE/PIB 16W	Relapse	BASELINE	NS3/4A	V170I	98.18		
							NS5A	Y93H	4.83		
						PTW 4	NS3/4A	V170I	99.91		
							NS5A	L31F	99.69	Y	
								Y93H	99.82		Y
	M14868-42362-1909	N	IFN- or P/R-exp	GLE/PIB 12W	Relapse	BASELINE	NS3/4A	V107I	41.5		
								V170I	99.83		
							NS5A	P29Q	2.17		
								A30K	99.6		
								E92*	2.65		
						PTW 8	NS3/4A	V107I	99.86		Y
								V170I	99.89		
							NS5A	A30K	99.79		
								E92*	2.5		
								Y93H	99.58	Y	
	M14868-43362-6801	N	IFN- or P/R-exp	GLE/PIB 12W	On-Tx VF	BASELINE	NS3/4A	A166S	96.96		
								V170I	99.88		
							NS5A	A30K	99.69		
						WEEK 8	NS3/4A	Y56H	95.5	Y	
								Y56N	4.26	Y	
								A166S	98.28		
								Q168L	69.03	Y	
								V170I	99.9		
							NS5A	P29Q	2.61	Y	
								A30K	99.78		
								P58Q	2.15	Y	
								P58T	2.07	Y	
								E92*	3.73	Y	
								Y93H	99.52	Y	
						PTW 24	NS3/4A	A166S	99.78		
								V170I	99.82		
							NS5A	A30K	99.79		
								Y93H	99.82	Y	
	M14868-43362-6809	Y	IFN- or P/R-exp	GLE/PIB 16W	On-Tx VF	BASELINE	NS3/4A	A166S	98.97		
								V170I	99.81		
							NS5A	Y93H	2.72		
						WEEK 8	NS3/4A	A156G	99.3	Y	
								A166S	98.18		
								V170I	99.9		
							NS5A	A30K	99.83	Y	
								E92*	2.3	Y	
								Y93H	99.65		Y
	M14868-45366-4806	Y	IFN- or P/R-exp	GLE/PIB 16W	Relapse	BASELINE	NS3/4A	Q80K	2.01		
								A166S	95.09		
								V170I	99.79		
						PTW 2	NS3/4A	Q80K	2.15		
								V170I	99.85		
							NS5A	M28G	97.5	Y	
								E92*	3.22	Y	
	M14868-47500-2809	N	IFN- or P/R-exp	GLE/PIB 16W	Relapse	BASELINE	NS3/4A	L132I	7.2		
								V170I	99.65		
							NS5A	A30K	99.75		
						PTW 4	NS3/4A	Y56H	99.35	Y	
								Q168R	99.81	Y	
								V170I	99.9		
							NS5A	A30K	99.87		
								Y93H	99.91	Y	
3b	M13594-47009-145301	N	Naïve	GLE/PIB 12W	Relapse	BASELINE	NS5A	V31M	99.79		
						PTW 2	NS3/4A	Q80K	99.04	Y	
							NS5A	V31M	99.84		
								Y93H	99.8	Y	

Appendix F: SVR12 rates in MAGELLAN-1 for GT1a and GT1b, NS5A inhibitor-experienced subjects according to presence of known resistance-associated substitutions at baseline (15% NGS cutoff).

Analysis includes NS5A inhibitor-experienced subjects (non-VF-censored) with or without prior PI experience.

HCV Subtype	Target	Position	Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
1a	NS3/4A	36	V36L		1/1		1/1
			V36M	0/1			0/1
			V36V/M	1/1			1/1
			V36L/M	**1/2 (50%)**	**1/1 (100%)**	**ND**	**2/3 (67%)**
		54	**T54S**	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		55	V55A	2/3			2/3
			V55I		1/1		1/1
			V55V/I	0/1			0/1
			V55A/I	**2/4 (50%)**	**1/1 (100%)**	**ND**	**3/5 (60%)**
		56	Y56H	1/1	0/1		1/2
			Y56Y/H		0/1		0/1
			Y56H	**1/1 (100%)**	**0/2 (0%)**	**ND**	**1/3 (33%)**
		122	S122G	1/1			1/1
			S122S/G		1/1		1/1
			S122G	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		132	**I132L**	**1/1 (100%)**	**ND**	**ND**	**1/1 (100%)**
		155	R155K	2/2	1/1		3/3
			R155R/K	0/1			0/1
			R155T	1/1			1/1
			R155K/T	**3/4 (75%)**	**1/1 (100%)**	**ND**	**4/5 (80%)**
		168	D168A		0/1		0/1
			D168D/A/T	0/1	0/1		0/2
			D168D/E		0/1		0/1
			D168D/V			1/1	1/1
			D168N	1/1			1/1
			D168V		1/1		1/1
			D168A/E/N/T/V	**1/2 (50%)**	**1/4 (25%)**	**1/1 (100%)**	**3/7 (43%)**
		170	**I170V**	**ND**	**3/3 (100%)**	**ND**	**3/3 (100%)**
	NS5A	24	K24Q		0/1		0/1
			K24R	3/3			3/3
			K24Q/R	**3/3 (100%)**	**0/1 (0%)**	**ND**	**3/4 (75%)**
		28	M28A		1/1		1/1
			M28A/V	1/1			1/1
			M28M/T	1/1			1/1
			M28M/T/V		0/1		0/1
			M28T	4/4	1/1	1/1	6/6
			M28V	1/2			1/2
			M28A/T/V	**7/8 (88%)**	**2/3 (67%)**	**1/1 (100%)**	**10/12 (83%)**
		30	Q30E	1/1			1/1
			Q30E/*	2/2			2/2
			Q30E/K/R	0/1			0/1
			Q30G/E	0/1			0/1
			Q30H	3/3	3/4	1/1	7/8
			Q30H/R			1/1	1/1
			Q30L			1/1	1/1
			Q30Q/E		1/1		1/1
			Q30Q/E/G	1/1			1/1
			Q30Q/H	3/3			3/3
			Q30Q/R	1/4	2/2	2/2	5/8
			Q30R	1/1	0/1	1/1	2/3

HCV Subtype	Target	Position	Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
			Q30R/L		0/1		0/1
			Q30E/G/H/K/L/R	**12/17 (71%)**	**6/9 (67%)**	**6/6 (100%)**	**24/32 (75%)**
		31	L31L/M	1/1			1/1
			L31L/V			1/1	1/1
			L31M	3/3	2/4	0/1	5/8
			L31M/V	**4/4 (100%)**	**2/4 (50%)**	**1/2 (50%)**	**7/10 (70%)**
		58	H58C	0/1			0/1
			H58D	0/1			0/1
			H58D/Y		1/1		1/1
			H58H/D			0/1	0/1
			H58H/Q		1/1		1/1
			H58H/Y	2/2			2/2
			H58P	3/3	1/1		4/4
			H58C/D/P/Q/Y	**5/7 (71%)**	**3/3 (100%)**	**0/1 (0%)**	**8/11 (73%)**
		93	Y93H		1/3	2/2	3/5
			Y93H/N	1/1	1/1		2/2
			Y93N	2/2	4/4	2/2	8/8
			Y93Y/H	1/1			1/1
			Y93Y/N	1/1			1/1
			Y93H/N	**5/5 (100%)**	**6/8 (75%)**	**4/4 (100%)**	**15/17 (88%)**
1b	NS3/4A	**54**	**T54S**	**ND**	**1/1 (100%)**	**ND**	**1/1 (100%)**
		56	**Y56F**	**0/1 (0%)**	**3/3 (100%)**	**ND**	**3/4 (75%)**
		122	**S122T**	**ND**	**1/1**	**ND**	**1/1 (100%)**
		132	V132I	2/2	2/2		4/4
			V132L		1/1		1/1
			V132I/L	**2/2 (100%)**	**3/3 (100%)**	**ND**	**5/5 (100%)**
		170	**V170I**	**3/3 (100%)**	**3/3 (100%)**	**ND**	**6/6 (100%)**
	NS5A	**28**	**L28M**	**0/1 (0%)**	**ND**	**ND**	**0/1 (0%)**
		30	R30K	1/1			1/1
			R30Q		1/1		1/1
			R30K/Q	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		31	L31I	1/1			1/1
			L31I/M	1/1			1/1
			L31M	4/4	1/1		5/5
			L31V		1/1		1/1
			L31I/M/V	**6/6 (100%)**	**2/2 (100%)**	**ND**	**8/8 (100%)**
		32	**P32-Deletion**	**0/1 (0%)**	**ND**	**ND**	**0/1 (0%)**
		58	P58P/S/*		1/1		1/1
			P58S/*	1/1			1/1
			P58S	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		92	**A92E**	**1/1 (100%)**	**ND**	**ND**	**1/1 (100%)**
		93	Y93H	4/4	4/4		8/8
			Y93S	1/1			1/1
			Y93H/S	**5/5 (100%)**	**4/4 (100%)**	**ND**	**9/9 (100%)**

Appendix G: SVR12 rates in MAGELLAN-1 for GT1a and GT1b, NS5A inhibitor-experienced subjects according to presence of known resistance-associated substitutions at baseline (2% NGS cutoff).

Analysis includes NS5A inhibitor-experienced subjects (non-VF-censored) with or without prior PI experience.
*indicates stop codon detected, presumably a sequencing error.

HCV Subtype	Target	Position	Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
1a	NS3/4A	36	V36L		1/1		1/1
			V36M	0/1			0/1
			V36V/M	1/1			1/1
			V36L/M	**1/2 (50%)**	**1/1 (100%)**	**ND**	**2/3 (67%)**
		54	**T54S**	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		55	V55A	2/3			2/3
			V55I		1/1		1/1
			V55V/I	0/1		1/1	1/2
			V55A/I	**2/4 (50%)**	**1/1 (100%)**	**1/1**	**4/6 (67%)**
		56	Y56H	1/1	0/1		1/2
			Y56Y/H	0/1	0/2		0/3
			Y56H	**1/2 (50%)**	**0/3 (0%)**	**ND**	**1/5 (20%)**
		122	S122G	1/1			1/1
			S122S/G		2/2		2/2
			S122G	**1/1 (100%)**	**2/2 (100%)**	**ND**	**3/3 (100%)**
		132	I132I/V	1/1			1/1
			I132L	1/1			1/1
			I132L/V	**2/2 (100%)**	**ND**	**ND**	**2/2 (100%)**
		155	R155K	2/2	1/1		3/3
			R155R/K	0/1		1/1	1/2
			R155R/T		0/1		0/1
			R155T	1/1			1/1
			R155K/T	**3/4 (75%)**	**1/2 (50%)**	**1/1 (100%)**	**5/7 (71%)**
		168	D168A		0/1		0/1
			D168D/A/T	0/1	0/1		0/2
			D168D/E		0/1		0/1
			D168D/V			1/1	1/1
			D168N	1/1			1/1
			D168V		1/1		1/1
			D168A/E/N/T/V	**1/2 (50%)**	**1/4 (25%)**	**1/1 (100%)**	**3/7 (43%)**
		170	**I170V**	**ND**	**3/3 (100%)**	**ND**	**3/3 (100%)**
	NS5A	24	K24K/E	0/1			0/1
			K24K/N	1/1		1/1	2/2
			K24Q		0/1		0/1
			K24R	3/3			3/3
			K24E/N/Q/R	**4/5 (80%)**	**0/1 (0%)**	**1/1 (100%)**	**5/7 (71%)**
		28	M28A		1/1		1/1
			M28A/V	1/1			1/1
			M28M/L/I		1/1		1/1
			M28M/T	1/1		2/2	3/3
			M28M/T/V		0/1		0/1
			M28M/V	1/3	1/1		2/4
			M28T	4/4	1/1	1/1	6/6
			M28V	1/2			1/2
			M28A/L/I/T/V	**8/11 (73%)**	**4/5 (80%)**	**3/3 (100%)**	**15/19 (79%)**
		29	**P29Q**	**ND**	**1/1 (100%)**	**ND**	**1/1 (100%)**
		30	Q30E	1/1			1/1
			Q30E/*	2/2			2/2

HCV Subtype	Target	Position	Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
			Q30E/K/R	0/1			0/1
			Q30G/E	0/1			0/1
			Q30H	3/3	3/4	1/1	7/8
			Q30H/R			1/1	1/1
			Q30L			1/1	1/1
			Q30Q/E	1/1	1/1		2/2
			Q30Q/E/G	1/1			1/1
			Q30Q/E/R		1/1		1/1
			Q30Q/H	3/3			3/3
			Q30Q/K			1/1	1/1
			Q30Q/R	2/5	3/3	2/2	7/10
			Q30Q/T	1/1			1/1
			Q30R	1/1	0/1	1/1	2/3
			Q30R/L		0/1		0/1
			Q30E/G/H/K/L/R/T	**15/20 (75%)**	**8/11 (73%)**	**7/7 (100%)**	**30/38 (79%)**
		31	L31L/M	1/1			1/1
			L31L/V			1/1	1/1
			L31M	3/3	2/4	0/1	5/8
			L31M/V	**4/4 (100%)**	**2/4 (50%)**	**1/2 (50%)**	**7/10 (70%)**
		58	H58C	0/1			0/1
			H58D	0/1			0/1
			H58D/Y		1/1		1/1
			H58H/D	1/1	0/1	0/1	1/3
			H58H/Q		1/1		1/1
			H58H/R	1/1			1/1
			H58H/Y	2/2			2/2
			H58P	3/3	1/1		4/4
			H58C/D/P/Q/R/Y	**7/9 (78%)**	**3/4 (75%)**	**0/1 (0%)**	**10/14 (71%)**
		93	Y93H		1/3	2/2	3/5
			Y93H/N	1/1	1/1		2/2
			Y93N	2/2	4/4	2/2	8/8
			Y93Y/H	1/1			1/1
			Y93Y/N	1/1	1/1		2/2
			Y93H/N	**5/5 (100%)**	**7/9 (78%)**	**4/4 (100%)**	**16/18 (89%)**
1b	NS3/4A	**54**	**T54S**	**ND**	**1/1 (100%)**	**ND**	**1/1 (100%)**
		56	**Y56F**	**0/1 (0%)**	**3/3 (100%)**	**ND**	**3/4 (75%)**
		122	**S122T**	**ND**	**1/1 (100%)**	**ND**	**1/1 (100%)**
		132	V132I	2/2	2/2		4/4
			V132L		1/1		1/1
			V132I/L	**2/2 (100%)**	**3/3 (100%)**	**ND**	**5/5 (100%)**
		168	D168D/E/Y		1/1		1/1
			D168D/Y	1/1			1/1
			D168E/Y	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		170	V170I	3/3	3/3	0/0	6/6
			V170V/I	0/0	1/1	0/0	1/1
			V170I	**3/3 (100%)**	**4/4 (100%)**	**ND**	**7/7 (100%)**
	NS5A	**28**	**L28M**	**0/1 (0%)**	**ND**	**ND**	**0/1 (0%)**
		29	**P29Q**	**1/1 (100%)**	**ND**	**ND**	**1/1 (100%)**
		30	R30K	1/1			1/1
			R30Q		1/1		1/1
			R30R/H	1/1			1/1
			R30R/Q/L		1/1		1/1
			R30H/K/L/Q	**2/2 (100%)**	**2/2 (100%)**	**ND**	**4/4 (100%)**
		31	L31I	1/1			1/1
			L31I/M	1/1			1/1

HCV Subtype	Target	Position	Substitution(s)	GLE/PIB 12W (A,C,D)	GLE/PIB 16W (E)	GLE/PIB+RBV 12W (B)	All Arms
			L31M	4/4	1/1		5/5
			L31V		1/1		1/1
			L31I/M/V	**6/6 (100%)**	**2/2 (100%)**	**ND**	**8/8 (100%)**
		32	**P32-Deletion**	**0/1 (0%)**	**ND**	**ND**	**0/1 (0%)**
		58	P58P/S/*		1/1		1/1
			P58S/*	1/1			1/1
			P58S	**1/1 (100%)**	**1/1 (100%)**	**ND**	**2/2 (100%)**
		92	**A92E**	**1/1 (100%)**	**ND**	**ND**	**1/1 (100%)**
		93	Y93H	4/4	4/4		8/8
			Y93S	1/1			1/1
			Y93H/S	**5/5 (100%)**	**4/4 (100%)**	**ND**	**9/9 (100%)**

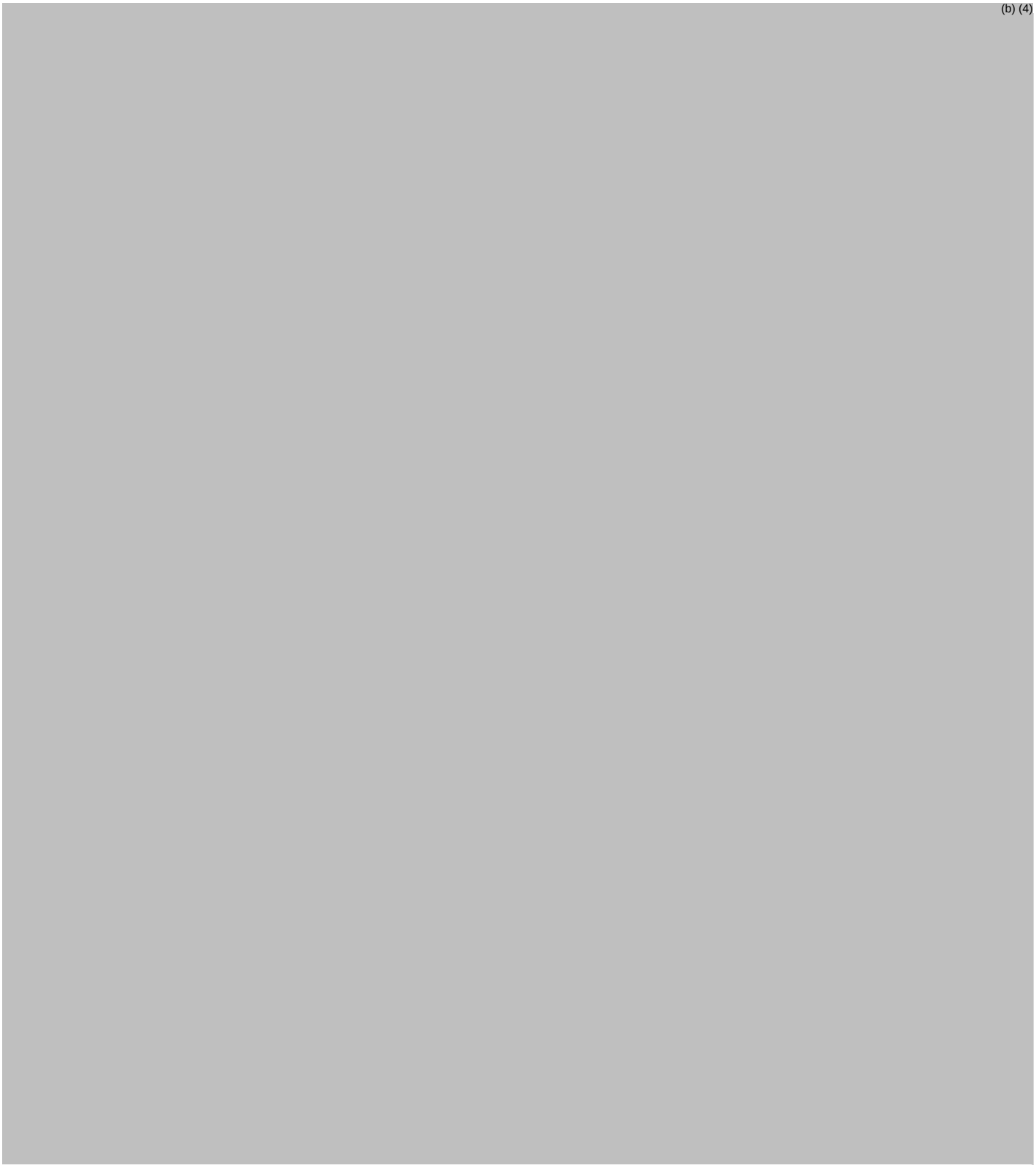
Appendix H: Individual subject-level map of NS3 and NS5A positions with detected baseline RASs for NS5A-experienced subjects who received GLE/PIB for 12 or 16 weeks in MAGELLAN-1 (2% NGS cutoff, non-VF censored).

Red indicates a substitution (relative to GT1a or GT1b reference) was detected at that position.

					NS3									NS5A										
SVR	Prior DAA Class Exp	Regimen	CIRR	USUBJID	36	54	55	56	122	132	155	168	170	24	28	29	30	31	32	58	92	93	# NS3 BL RAS	# NS5A BL RAS
N	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-53286-2924	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	2
			Y	M15410-38627-1321	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	3
		GP 16W	Y	M15410-42371-2021	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2
	NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-40621-3023	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-41723-2605	0	0	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0	2	3
			N	M15410-41723-2624	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	3	2
		GP 16W	N	M15410-41723-2622	0	0	0	1	0	0	0	1	0	0	1	0	1	1	0	1	0	0	2	4
			Y	M15410-12641-1620	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	2	2
			Y	M15410-40621-3025	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	3	2
Y	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-22887-2120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
			N	M15410-22887-2122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2
			N	M15410-37834-2401	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
			N	M15410-38627-1322	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
			N	M15410-38853-2223	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	4
			N	M15410-40621-3027	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	2
			N	M15410-41723-2602	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
			N	M15410-42362-1520	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
			N	M15410-50807-2801	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
			N	M15410-53286-2920	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
			N	M15410-41723-2620	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Y	M15410-37759-4526	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2
			Y	M15410-53286-2922	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2
		GP 16W	N	M15410-37759-4527	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2
			N	M15410-38627-1320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
			N	M15410-38853-2220	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
			N	M15410-38853-2222	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
			N	M15410-40621-3024	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	2
			N	M15410-41723-2621	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1
			N	M15410-53286-2921	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
			N	M15410-76269-4820	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-40621-3031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			N	M15410-40622-5020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Y	M15410-42730-1025	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	2
			Y	M15410-76269-4821	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1
	NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-18161-1208	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
			N	M15410-12641-1619	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
			N	M15410-12641-1622	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2
			N	M15410-18161-1221	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
			N	M15410-35827-1901	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	4
			N	M15410-37739-1805	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-37739-1806	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2
			N	M15410-37759-4521	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
			N	M15410-38627-1305	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	2
			N	M15410-40621-3030	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
			N	M15410-40622-5022	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-51321-4422	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	4	1
			N	M15410-51382-1402	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
			N	M15410-40621-3022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			N	M15410-44319-3725	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Y	M15410-44318-3623	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	2	1
		GP 16W	N	M15410-12641-1621	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-37739-1820	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	2
			N	M15410-40621-3021	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
			N	M15410-42730-1024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
			N	M15410-44319-3724	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	2	2
			N	M15410-44372-4020	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	2	2
			N	M15410-92059-3420	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
			N	M15410-41723-2623	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-44372-4021	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	2
Y	NS5A I-Exp, NS/4A PI-Naïve	GP 12W	N	M15410-38645-2501	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
			Y	M15410-22887-2125	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	1	3
			Y	M15410-38853-2225	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	2
			Y	M15410-40621-3020	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
			Y	M15410-40724-3921	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
		GP 16W	N	M15410-38853-2226	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1
			N	M15410-47163-3823	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	4	1
	NS3/4A PI- and NS5A I-Exp	GP 12W	N	M15410-18161-1209	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1
			N	M15410-51382-1401	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1
			N	M15410-40724-3923	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0	1	2	3
		GP 16W	N	M15410-40621-3032	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0
			N	M15410-44319-3720	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	1	3	3
			N	M15410-44319-3722	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
			Y	M15410-40724-3922	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	3	2

Appendix I: Selected slides from internal discussion on key Midcycle review issues

(b) (4)



2 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

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/s/

PATRICK R HARRINGTON
05/10/2017

JULIAN J O REAR
05/11/2017