Hepatitis C virus (HCV) protease inhibitors combined with pegylated alfa interferon-ribavirin have demonstrated improved efficacy compared with pegylated alfa interferon-ribavirin alone for the treatment of chronic hepatitis C. Asunaprevir (BMS-650032), a novel HCV NS3 protease inhibitor in clinical development, was evaluated for safety, antiviral activity, and resistance in four double-blind, placebo-controlled, sequential-panel, single- and multiple-ascending-dose (SAD and MAD) studies in healthy subjects or subjects with chronic HCV genotype 1 infection. In SAD studies, subjects (healthy or with chronic HCV infection) were randomized to receive asunaprevir in dose groups of 10 to 1,200 mg or a placebo. In MAD studies, healthy subjects were randomized to receive asunaprevir in dose groups of 10 to 600 mg twice daily or a placebo for 14 days; subjects with HCV infection received asunaprevir in dose groups of 200 to 600 mg twice daily, or a placebo, for 3 days. Across all four studies, headache and diarrhea were the most frequent adverse events in asunaprevir recipients. Asunaprevir at doses of 200 to 600 mg resulted in rapid HCV RNA decreases from the baseline; maximal mean changes in HCV RNA over time were 2.7 and 3.5 log_{10} IU/ml in the SAD and MAD studies, respectively. No enrichment of signature asunaprevir-resistant viral variants was detected. In conclusion, the novel NS3 protease inhibitor asunaprevir, when administered at single or multiple doses of 200 to 600 mg twice daily, is generally well tolerated, achieving rapid and substantial decreases in HCV RNA levels in subjects chronically infected with genotype 1 HCV.

The introduction of long-acting alfa peginterferon (alfa) was an important advance in the treatment of chronic hepatitis C virus (HCV) infection. In combination with ribavirin (RBV), alfa provides overall sustained virologic response (SVR) rates of approximately 40% in treatment-naive patients infected with HCV genotype 1 and 80% in patients with genotype 2 or 3 (12). For genotype 1 infections, combining alfa-RBV with one of the recently approved NS3 protease inhibitors, telaprevir or boceprevir, can provide higher SVR rates than alfa-RBV alone in both HCV treatment-naive patients (5, 15) and those previously treated with alfa-RBV (2, 21). Although these improvements are encouraging, initial results with multiple new agents in development suggest that further advances in both efficacy and tolerability can be anticipated (6).

Potential advances in the treatment of HCV infection include novel immunologic modulators and small-molecule-based inhibitors of HCV replication (11). Among antiviral agents under development is a novel HCV NS3 protease inhibitor, asunaprevir (BMS-650032; http://www.ama-assn.org/resources/doc/usan/asunaprevir.pdf) (13, 14). Asunaprevir is a potent inhibitor of HCV replication in the replicon system, with 50% effective concentrations (EC_{50}) of 4.0 nM and 1.0 nM against genotypes 1a and 1b, respectively (14).

We report the results of the exploratory clinical development program to evaluate the safety and tolerability, antiviral activity, and resistance profile of asunaprevir. The results of these single- and multiple-ascending-dose studies in healthy subjects or subjects chronically infected with HCV genotype 1 will guide the future clinical development program of asunaprevir. These studies used similar protocols and analytic methods and are reported together to provide a larger data set for evaluation of key safety and viral parameters. Pharmacokinetic data derived from these studies were complex and will be reported separately.

**MATERIALS AND METHODS**

**Study designs.** This report concerns four double-blind, placebo-controlled, sequential-panel, single-ascending-dose (SAD) or multiple-ascending-dose (MAD) studies in healthy subjects and subjects with chronic HCV infection.

(i) SAD-HS. In the double-blind, placebo-controlled, sequential SAD study in healthy subjects (SAD-HS), eight healthy subjects were enrolled in each of seven dose panels (10, 50, 100, 200, 400, 600, and 1,200 mg). Subjects were admitted to the clinical facility 2 days prior to dosing (day −2). On day 1, subjects were randomly assigned in a ratio of 3:1 to receive a single oral dose of asunaprevir (n = 6/dose group) or placebo (n = 2). Subjects remained in the clinical facility for at least 72 h after dosing and...
were furloughed from the clinical facility on day 4 and discharged on day 7 following a safety assessment.

(ii) SAD-cHCV. In the double-blind, placebo-controlled, sequential SAD study in subjects with chronic HCV genotype 1 (SAD-cHCV), six subjects with chronic HCV infection were enrolled in each of four sequential dose panels (10, 50, 200, and 600 mg asunaprevir). The 600-mg dose was selected as the final dose based upon data from the preceding dose panels. Subjects were admitted to the clinical facility on day −1 (the day prior to dosing). On day 1, subjects were randomly assigned in a ratio of 5:1 to receive a single oral dose of asunaprevir (n = 5/dose group) or placebo (n = 1). Forty-eight hours after dosing (day 3), subjects were furloughed from the facility; they had an outpatient visit on day 4 and were discharged on day 7 following a safety assessment.

(iii) MAD-HS. In the double-blind, placebo-controlled, sequential MAD study in healthy subjects (MAD-HS), eight healthy subjects were enrolled in each of six dose panels (10, 50, 100, 200, 400, and 600 mg asunaprevir every 12 h). Subjects were admitted to the clinical facility on day −1. On day 1, participants were randomly assigned in a ratio of 3:1 to receive asunaprevir (n = 6/dose group) or placebo (n = 2) every 12 h for 14 days. Asunaprevir was dosed twice daily in both MAD studies on the basis of the frequent viral rebound prior to 24 h that was observed in the SAD-cHCV study (see Results and Fig. 1). Subjects remained in the clinical facility for at least 72 h after the final dosing on the morning of day 14 and were furloughed on day 17 and discharged on day 21 following a safety assessment.

(iv) MAD-cHCV. In the double-blind, placebo-controlled, sequential MAD study in subjects with chronic HCV genotype 1 (MAD-cHCV), five HCV-infected subjects were randomly assigned within each of three dose panels (200, 400, and 600 mg asunaprevir every 12 h). Subjects were admitted to the clinical facility on day −1. On day 1, subjects were randomly assigned in a ratio of 4:1 to receive asunaprevir (n = 4/dose group) or placebo (n = 1) every 12 h for 3 days. Subjects remained in the clinical facility from day −1 until the morning of day 4. Subjects returned for additional blood sampling for clinical laboratory tests, pharmacokinetic and/or viral RNA measurements, and resistance testing at approximately days 5 to 10, 14, 21, and 28. Additional longer-term follow-up visits were scheduled at days 42, 98, and 182.

In all studies, escalation to the subsequent dose panel took place only after safety data from the previous panel had been analyzed and dose escalation was deemed safe by the sponsor and the investigators. Subjects who did not complete the study (except those whose participation was discontinued for adverse events) could be replaced. The morning dose on the first and last days of dosing required an overnight fast; all other doses in every 12-h (Q12h) dosing regimen (MAD studies) required fasting for at least 2 h before and approximately 2 h after dosing.

Sample sizes. The number of subjects in each study was based on primary assessments in the respective studies: safety and tolerability in the two SAD studies and the MAD study in healthy subjects and changes in log_{10} HCV RNA from the baseline to day 3 in the MAD-cHCV study. Accordingly, five or six subjects administered active drug at each dose level in the two SAD studies and the MAD-HS study provided 80% probability of observing at least one occurrence at a dose level of any adverse event that would occur with 28% or 24% incidence, respectively, in the population from which the sample was drawn. Active drug was administered to four subjects per dose level in the MAD-cHCV study, providing probabilities of 0.01 and 0.04 to observe a mean decrease from the baseline of ≥1.5 log_{10} HCV RNA if the true mean decrease is 0.0 and 2.5 log_{10} HCV RNA, respectively. This calculation was based on the assumption that the decrease in log_{10} HCV RNA from the baseline to day 3 is normally distributed and that the standard deviation for the decrease in log_{10} HCV RNA from the baseline to day 3 is 1.3.

These studies were approved by institutional review boards in all study centers and conducted in accordance with good clinical practice, as defined by the International Conference on Harmonization, in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the U.S. Code of Federal Regulations, Title 21, Part 50, and in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Informed written consent was obtained from all subjects.

Subject randomization. Within each dose panel for each study, patients were randomly assigned to receive asunaprevir or a placebo according to a computer-generated randomization scheme prepared by Bristol-Myers Squibb. An interactive voice response system was used to assign a unique subject number and a blinded container number, which was provided to the blinded study staff who supervised and recorded the drug administration.

Inclusion and exclusion criteria. Healthy subjects were identified by medical history, physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory evaluations. Men and women ages 18 to 49 years with a body mass index of 18 to 32 kg/m² were eligible to participate. Female participants could not be nursing, pregnant, or of childbearing potential. Eligible patients with chronic HCV infection were men or women aged 18 to 60 years with a body mass index of 18 to 35 kg/m² and chronic infection with HCV genotype 1, either treatment naive, treatment nonresponders (including relapsers), or treatment intolerant. Additional inclusion criteria were plasma HCV RNA levels of ≥100,000 IU/ml, a documented FIB-4 score of ≤0.72 or ≤0.59, and an aspartate aminotransferase platelet ratio index of ≤2 or the absence of cirrhosis based on liver biopsy within 12 months. Main exclusion criteria included previous exposure to another NS3 protease inhibitor, coinfection with human immunodeficiency virus or hepatitis B virus, or being women of childbearing potential.

Safety and antiviral evaluations. Blood and urine samples for clinical laboratory evaluations, single 12-lead ECG, and vital sign measurements were collected at specified time points throughout all studies. Safety assessments were based on reported adverse events (AEs) and the results of vital sign measurements, physical examinations, ECGs, and clinical laboratory tests. The incidences of AEs were tabulated and reviewed for potential significance and clinical importance.

In the SAD studies, 12-lead ECGs were recorded at screening, 24 h predosing, and at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h postdosing and prior to discharge. In the MAD-HS study, ECGs were recorded at screening, day −1 and 1 h after morning dosing on days 2, 4, 6, 9, 11, 13, 17, and 21. Additional serial ECGs were recorded 0 (predose), 1, 2, 4, 6, 8, and 12 h postdosing on days −1, 1, and 14 and 0, 2, and 4 h after morning dosing on days 3 and 7. In the MAD-cHCV study, ECGs were recorded at screening and at 0 (predose), 2, 4, 8, and 12 h postdosing on day 1, and 1 h after morning dosing on days 2, 3, 4, 7, 14, 21, and 28.

Antiviral response was assessed by the magnitude of change in plasma HCV RNA levels from baseline. The primary assessment for antiviral activity in the MAD study was a decrease in plasma HCV RNA levels from baseline to day 3. HCV RNA levels were determined using the Roche Cobas TaqMan HCV test, v2.0 (lower limit of quantification, 25 IU/ml; lower limit of detection, 10 IU/ml).

Resistance analyses. In studies of HCV-infected subjects, treatment-emergent HCV variants were analyzed genotypically by population sequencing of the NS3 protease domain; samples with sequences that changed during treatment or contained variants predicted to confer resistance were subsequently phenotyped if HCV RNA was ≥1,000 IU/ml. In the SAD-cHCV study, genotypic analysis was conducted on samples collected at baseline and 1, 2, and 6 days postdosing from subjects receiving 200-mg or 600-mg doses of asunaprevir and at baseline only in subjects receiving 10-mg or 50-mg doses of asunaprevir. In the MAD-cHCV study, genotypic analysis was conducted on samples collected at baseline, day 4 (12 h after the final dose) and day 6 from all asunaprevir recipients. The 4-h sample was used as a baseline for two subjects with missing baseline samples in the 400-mg cohort. For subjects with emergent polymorphisms detected at day 6, genotypic analyses were also conducted at days 42 and 182.

HCV RNA was isolated from subject serum with a QIAamp MinElute...
TABLE 1 Baseline characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy subjects, single ascending dose</td>
</tr>
<tr>
<td></td>
<td>Asunaprevir</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>42</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>100</td>
</tr>
<tr>
<td>Age, mean yrs (SD)</td>
<td>31.9 (6.71)</td>
</tr>
<tr>
<td>Race, % white</td>
<td>28.6</td>
</tr>
<tr>
<td>BMI, mean kg/m² (SD)</td>
<td>26.4 (2.58)</td>
</tr>
<tr>
<td>HCV treatment naive, %</td>
<td>55</td>
</tr>
<tr>
<td>HCV genotype, % 1a</td>
<td>85</td>
</tr>
</tbody>
</table>

*a The placebo group excludes 2 subjects who received ritonavir in a subsequent crossover design as part of exploratory pharmacokinetic analyses, not reported here.

**TABLE 1**

**Viral vacuum kit (Qiagen, Inc., Valencia, CA).** First-strand cDNA was synthesized from random hexamer primers with a SuperScript III first-strand synthesis system for reverse transcriptase PCR (Invitrogen Corp., Carlsbad, CA). The NS3 protease-coding region was amplified with genotype-specific primers. At least two independent PCR products were amplified with different primer sets for each sample. A second PCR with the same primers or a nested PCR with internal primers was performed when required to obtain sufficient NS3 protease cDNA for sequence analysis. Sequences covering both strands were obtained for purified PCR products and compared with reference HCV genotype 1a (H77c) or genotype 1b (Con1) sequences.

**Statistical analysis.** All recorded adverse events were listed and tabulated by system organ class, preferred term, and treatment. Vital signs and clinical laboratory tests were listed and summarized by treatment. Any significant physical exam findings and clinical laboratory results were listed. The effects of asunaprevir on ECG parameters were explored graphically and by summary statistics.

The magnitude of changes in plasma HCV RNA levels was assessed by summarizing changes from baseline (day −1) by time (or study day) and treatment, summarizing maximum observed changes from baseline by treatment; the primary assessment of antiviral activity was based on the change from baseline to day 3 for the MAD-cHCV study. All statistical analyses were carried out using the software program SAS/STAT version 8.2 (SAS Institute Inc., Cary, NC).

**RESULTS**

**Participant disposition and baseline characteristics.** Baseline characteristics for enrolled subjects are summarized in Table 1. In the SAD-HS study, 56 subjects were enrolled in seven treatment groups as described in Materials and Methods; 55 subjects completed the study, and 1 subject was discontinued due to poor compliance. In the MAD-HS study, 48 subjects were enrolled in seven treatment groups (six asunaprevir dose groups and a placebo group); 46 subjects completed the study, and two subjects discontinued: one withdrew consent, and one withdrew following on-treatment discovery of human immunodeficiency virus infection that met exclusion criteria. In the SAD study in subjects with chronic HCV genotype 1, 24 patients were enrolled in four treatment groups; all 24 patients completed the study. In the MAD-cHCV study, 15 patients were enrolled in three treatment groups; 10 patients completed the study. Four patients withdrew consent, and one patient died 18 days following the last dose of asunapre...
(QTcF) or of changes in QTcF versus time after dosing (ΔQTcF; data not shown).

Resistance analysis. Variants at NS3 amino acid position 155 or 168 have been shown to confer high-level resistance to NS3 protease inhibitors (16). In an in vitro HCV genotype 1a (H77c) replicon system, the predominant asunaprevir-resistant variant emerged at NS3-R155K and was shown to confer a 26-fold loss of asunaprevir potency compared with that for wild-type genotype

![Image 1](attached_image_1)

**FIG 1** HCV RNA response, SAD-cHCV study. Changes from baseline are shown in serum hepatitis C virus (HCV) RNA following a single 10-mg to 600-mg dose of asunaprevir in the single-ascending-dose study in HCV-infected subjects. Dashed lines indicate data for individual subjects with HCV genotype 1a (blue) or 1b (red) infection; solid black lines indicate means (placebo, n = 4; ASV groups, n = 5/group).

![Image 2](attached_image_2)

**FIG 2** HCV RNA response, MAD-cHCV study. Changes from baseline are shown in serum HCV RNA following twice daily 200-mg to 600-mg doses of asunaprevir for 3 days in the multiple-ascending-dose study in HCV-infected subjects. The shaded area indicates the dosing period. Dashed lines indicate data for individual subjects with HCV genotype 1a (blue) or 1b (red) infection; solid black lines indicate means (placebo, n = 3; ASV groups, n = 4/group).
Analyses of subject-derived NS3 protease sequences indicated that inhibitors (10), was detected by population sequencing in baseline has been associated with low-level resistance to NS3 protease in established observations. The 1a-NS3-Q80K polymorphism, which sequenced were found in 77% to 100% of molecular clones. Clonal analysis revealed that none conferred resistance when assessed in an NS3 protease chimeric replicon cell-based phenotypic assay. Furthermore, all four subjects demonstrated on-treatment decreases in HCV RNA that were consistent with others in their respective dose groups (Fig. 4). Maximum declines in serum HCV RNA were 2.07, 2.92, 3.39, and 2.21 log10 IU/ml for subjects 1 to 4, respectively. Except for the NS3-Q80K polymorphism, no known resistance polymorphisms (at NS3 position 36, 43, 54, 55, 122, 155, 156, 168, or 170) were detected in the four subjects examined by clonal analysis (14).

In the SAD-cHCV study, virologic responses were minimal in subjects who received 10-mg or 30-mg doses of asunaprevir, and only baseline samples were sequenced. In these subjects, polymorphisms were detected at sites known to confer low-level resistance; susceptibility analysis of the subject-derived NS3 protease sequences did not appear to confer resistance in full-length NS3/4A protease enzyme-based susceptibility assays (50% inhibitory concentrations [IC50] ranging from 0.91 to 2.0 nM for genotype 1a-infected subjects, versus 1.3 nM when asunaprevir was tested against 1a [H77c], and 0.53 nM for a genotype 1b-infected subject, versus 0.55 nM for 1b [Con1]). The resistance variant 1b-NS3E168 was identified in a placebo recipient at all time points analyzed; in susceptibility assays, the NS3 protease sequence derived from this subject conferred 57-fold resistance to asunaprevir (13). In population sequencing analysis, two subjects, one each in the clones at day 6 and persisted at day 42. Population sequencing revealed the continued persistence of this polymorphism at the last time point examined (day 182). Phenotypic analysis of the treatment-emergent polymorphisms that were detected in all four subjects revealed that one conferred resistance when assessed in an NS3 protease chimeric replicon cell-based phenotypic assay.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Adverse events occurring in &gt;2 subjects in studies of healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of subjects with adverse event</td>
<td>Multiple-ascending-dose cohort</td>
</tr>
<tr>
<td>Single-ascending-dose cohortb</td>
<td>Asunaprevir treatment</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
</tr>
<tr>
<td>Headache 10</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>(18.4)</td>
</tr>
<tr>
<td>Diarrhea 1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

a All other adverse events occurred in no more than one individual, combining all dose groups in both studies.

b n = 6 for each dose group (n = 42 total for the asunaprevir cohort).

c n = 6 for each dose group (n = 42 total for the asunaprevir cohort); n = 12 for the placebo group.

d n = 6 for each dose group (36 total for the asunaprevir cohort).

e n = 6 for each dose group (36 total for the asunaprevir cohort).

1a (H77c) HCV (14). In an in vitro HCV genotype 1b (Con1) replicon, NS3-D168V emerged as the predominant resistant variant, conferring a 415-fold loss of asunaprevir potency. In the MAD-cHCV study, R155 and D168 substitutions were not detected by population sequencing in either baseline or on-treatment samples. For one subject infected with HCV genotype 1b, an NS3 polymorphism was detected at baseline at amino acid position 122 (S122R), which has been associated with NS3 protease inhibitor resistance (20), but the sequence was susceptible to asunaprevir inhibition in chimeric replicon susceptibility analysis (EC50 = 6.6 nM, versus 3.8 nM for genotype 1b [Con1]). The resistance variant 1b-NS3-D168V emerged as the predominant resistant variant. Furthermore, all four subjects demonstrated on-treatment decreases in HCV RNA that were consistent with others in their respective dose groups (Fig. 4). Maximum declines in serum HCV RNA were 2.07, 2.92, 3.39, and 2.21 log10 IU/ml for subjects 1 to 4, respectively. Except for the NS3-Q80K polymorphism, no known resistance polymorphisms (at NS3 position 36, 43, 54, 55, 122, 155, 156, 168, or 170) were detected in the four subjects examined by clonal analysis (14).

Changes in sequence polymorphisms were observed in four asunaprevir recipients over time (Fig. 3). Clonal analysis revealed that the polymorphisms identified as single species by population sequencing were found in 77% to 100% of molecular clones (n = 26 to 35) examined at all tested time points. Clonal analysis also confirmed that samples identified as mixtures by population sequencing had higher proportions of the minority variants. In three of these four subjects, the changes observed at day 4 reverted to the baseline genotype at day 6 (i.e., 2 days posttreatment). In the fourth subject, the Q80K polymorphism was found in 96% of the placebo group (n = 12) excludes 2 subjects who received ritonavir in a subsequent crossover design as part of exploratory pharmacokinetic analyses, not reported here.

d n = 6 for each dose group (36 total for the asunaprevir cohort).

e n = 6 for each dose group (36 total for the asunaprevir cohort).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Adverse events occurring in &gt;1 subject in studies of HCV-infected subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects with adverse event</td>
<td>Placebo</td>
</tr>
<tr>
<td>Single-ascending-dose cohortb</td>
<td>Asunaprevir dose (mg)</td>
</tr>
<tr>
<td>Asunaprevir dose (mg)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Headache 10</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Flatulence 1</td>
<td>1</td>
</tr>
<tr>
<td>Nausea 1</td>
<td>1</td>
</tr>
</tbody>
</table>
asunaprevir EC<sub>50</sub> values. Treatment-emergent changes in NS3 sequence were evidence of cardiotoxicity (18, 19), a detailed analysis of cardiac human immunodeficiency virus protease inhibitors have shown short-term monotherapy. Because some inhibitors of HCV and does not increase the incidence of either rash or anemia with ily managed. In particular, these studies suggest that asunaprevir discontinuation or modification and were generally mild and eas-

verse polymorphism exists at NS3 position 80; HCV genotype 1a Q80K polymorphism, which has been associated with low-

DISCUSSION

The potential value of HCV NS3 protease inhibitors in the combi-
tination treatment of chronic HCV infection was confirmed in recent large trials in treatment-naive and treatment-expe-
priate patients with HCV genotype 1 infection (2, 4, 5, 15, 21). Combining telaprevir or boceprevir with alfa-RBV improved efficacy over that with alfa-RBV alone but also increased AEs, particularly skin rash (telaprevir) and anemia (boceprevir), resulting in more treat-
ment discontinuations in the triple-combination groups (2, 5, 15, 21). Thus, these trials confirmed that adding an HCV protease inhibitor to alfa-RBV increases SVR rates over those achieved with alfa-RBV alone but also demonstrated the need for development of alternative protease inhibitors with more favorable safety pro-
files.

The AE profile of asunaprevir indicates that the drug was generally well tolerated in SAD and MAD studies in healthy volun-
teers for up to 14 days and for up to 3 days in subjects with chronic HCV infection. While the individual studies were small and in-
volved monotherapy exposure limited to a maximum of 14 days, the AEs observed in asunaprevir recipients did not require dose discontinuation or modification and were generally mild and easily managed. In particular, these studies suggest that asunaprevir does not increase the incidence of either rash or anemia with short-term monotherapy. Because some inhibitors of HCV and human immunodeficiency virus protease inhibitors have shown evidence of cardiotoxicity (18, 19), a detailed analysis of cardiac safety was undertaken. Detailed ECG analysis provided no evi-
dence of an increased risk of cardiac adverse events associated with asunaprevir. These findings are encouraging but must be regarded as preliminary, since longer-term dosing with asunaprevir in combination with other agents is likely to be necessary to achieve sustained clearance of HCV infection, and the safety profile of asunaprevir must be confirmed in that setting.

Asunaprevir administration resulted in a prompt and clinically relevant antiviral effect after single- and multiple-dose adminis-
tration. The effect of asunaprevir on HCV RNA levels following single or multiple doses of drug is consistent with the well-de-
scribed critical role of HCV NS3 protease in viral replication (1, 3, 8). Inhibition of the NS3 protease blocks the release of nonstruc-
tural proteins required for HCV replication (8). This mechanism of action occurs at a point earlier in the HCV replicative life cycle than that of agents that act directly on HCV RNA replication (for example, polymerase inhibitors) and differs fundamentally from the immunomodulatory and antiviral effects of alfa-RBV (8). Asunaprevir elicited rapid mean reductions in HCV RNA from baseline of up to 3.7 log<sub>10</sub> IU/ml at day 3; these changes are well within the ranges reported for other direct-acting antiviral (DAA) agents (2, 4, 5, 15, 21).

The results of resistance analyses in subjects treated with asu-
aprevir revealed the emergence of variants that did not signifi-
cantly impact phenotypic susceptibility. Enrichment of variants reported to confer resistance to asunaprevir in vitro was not de-
tected by population sequencing or clonal analysis (14). These results suggest that twice-daily doses of asunaprevir of ≥200 mg may be sufficient to suppress replication of drug-resistant variants to below the level of detection over treatment durations of up to 3 days, in contrast to a similar study with another NS3 protease inhibitor that showed rapid emergence of drug-resistant variants during short-term treatment (9). However, further studies are needed to determine the frequency with which drug-resistant variants may emerge with longer-term treatment.

Preexisting HCV resistance variants were detected in baseline samples from some subjects; the most frequent of these is the NS3-Q80K polymorphism, which has been associated with low-
level resistance to other HCV NS3 protease inhibitors (10). A nat-
ural polymorphism exists at NS3 position 80; HCV genotype 1a sequences in the database are divided approximately equally be-

FIG 3 Phenotypic effect of treatment-emergent NS3 sequence changes on asunaprevir EC<sub>50</sub> values. Treatment-emergent changes in NS3 sequence were detected in four subjects, all infected with HCV genotype 1a, in the multiple-

FIG 4 HCV RNA response in subjects with treatment-emergent changes in NS3 protease sequence. Individual HCV RNA changes over time are shown for the four asunaprevir recipients (all HCV genotype 1a) with treatment-emer-
gen changes in NS3 protease sequences in the multiple-ascending-dose study in HCV-infected subjects.

200-mg and 600-mg dose groups, exhibited multiple time-related NS3 sequence polymorphisms. Only one of these changes, 1a-

ns-NS3-Q80K, identified in the 600-mg recipient, has been associ-
ated with resistance to NS3 protease inhibitors (10), and none of these changes affected susceptibility to asunaprevir in phenotypic analysis (13).
significantly impact phenotypic susceptibility to asunaprevir, its effects during long-term therapy are yet to be determined. The only detectable preexisting variant of potential significance is NS3-E168, since this has been reported to confer resistance to asunaprevir (13). The presence of such a polymorphism prior to treatment may limit virologic response with longer-term monotherapy, suggesting that similar to the case with other NS3 protease inhibitors, combining asunaprevir with peginterferon and RBV and/or DAAs with alternative mechanisms of action will be needed to suppress the emergence of resistance. This will need to be explored in longer-term combination trials.

In conclusion, in SAD and MAD studies in healthy subjects and subjects with chronic HCV infection, the orally available NS3 protease inhibitor asunaprevir was generally well tolerated and demonstrated antiviral responses supportive of once- or twice-daily dosing. These studies have confirmed the robust antiviral effect of asunaprevir, based on the well-described mechanism of NS3 protease inhibition. As therapy for chronic HCV evolves to a paradigm of multiple drug combinations, the safety profile and antiviral effects observed to date support further studies of asunaprevir in combination with pegylated interferons and ribavirin, as well as other investigational DAAs or immunomodulators.

ACKNOWLEDGMENTS

The studies included in this report were funded by Bristol-Myers Squibb. Editorial assistance for preparing this report was provided by Richard Boehme of Articulate Sciences and funded by Bristol-Myers Squibb.

We thank Min Lee, Susan Chaniewski, Paul Falk, and Fei Yu for resistance testing technical support.

REFERENCES