Grazoprevir, Elbasvir, and Ribavirin for Chronic Hepatitis C Virus Genotype 1 Infection After Failure of Pegylated Interferon and Ribavirin With an Earlier-Generation Protease Inhibitor: Final 24-Week Results From C-SALVAGE

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Background. The phase 2 C-SALVAGE study (Hepatitis C-Salvage Study for Patients who Failed DAA/PR Therapy) demonstrated a 96.2% sustained virologic response at 12 weeks (SVR12) rate using the NS3/4A protease inhibitor grazoprevir and the NS5A inhibitor elbasvir together with ribavirin in treatment-experienced patients with chronic hepatitis C virus (HCV) genotype 1 infection.

Methods. C-SALVAGE was a prospective open-label trial of grazoprevir 100 mg once daily and elbasvir 50 mg once daily coadministered with weight-based ribavirin twice daily for 12 weeks in genotype 1–infected cirrhotic and noncirrhotic patients who had failed treatment with ≥4 weeks of pegylated interferon and ribavirin plus either boceprevir, telaprevir, or simeprevir. Although the primary efficacy outcome was SVR12, patients were also evaluated 24 weeks after cessation of study therapy. Population sequencing was performed at baseline and periodically in virologic failures throughout the 24-week posttherapy follow-up period.

Results. SVR24 rates were 76 of 79 (96.2%) overall, with all 3 relapses occurring by posttherapy week 8. Every NS3 and NS5A variant detected at baseline reappeared at the time of relapse and persisted throughout the available follow-up period. NS3_A156T emerged in virus from each patient at relapse, but rapidly disappeared over the ensuing 2 weeks in 2 patients. NS5A_Y93H emerged in virus from 2 patients at relapse and persisted for the entire follow-up period.

Conclusions. Grazoprevir and elbasvir with ribavirin for 12 weeks maintained HCV suppression for at least 24 weeks posttherapy without late relapses. Baseline resistance-associated variants (RAVs) stably reappeared at relapse in all 3 patients with virologic failure. NS5A_RAVs emerging at relapse persisted for the full 24-week follow-up period. If confirmed, this finding could complicate retreatment of the small number of patients failing regimens containing an NS5A inhibitor.

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Keywords. C-SALVAGE; genotype 1; grazoprevir; elbasvir; resistance-associated variants (RAVs).

Virologic failure during or after treatment with direct-acting antiviral agents (DAAs) is typically accompanied by the emergence of resistance-associated variants (RAVs) [1,2]. Additional non-cross-resistant drugs are needed for salvage therapy of patients with chronic hepatitis C virus (HCV) infection who do not achieve sustained virologic response (SVR) on DAA regimens [1]. NS3 variants conferring high-level resistance to the first-generation protease inhibitors (PIs) are well recognized, but the degree of cross-resistance between older and newer drugs in the same class is an active area of investigation [1–7].

The C-SALVAGE study (Hepatitis C-Salvage Study for Patients who Failed DAA/PR Therapy) investigated an interferon-free combination of grazoprevir (an NS3/4A PI) and elbasvir (an NS5A inhibitor) with ribavirin for patients with chronic HCV genotype 1 infection who had previously failed triple therapy with pegylated interferon and ribavirin (PR) plus an earlier-generation PI [8]. In this open-label trial, 79 patients were retreated with grazoprevir and elbasvir plus ribavirin after failure of combination therapy with PR plus either boceprevir, telaprevir, or simeprevir. Despite a high prevalence of NS3 variants at baseline, the overall SVR rate at 12 weeks (SVR12) was 96.2% (76/79) due to 3 patients relapsing during the initial 8 weeks of posttherapy follow-up coincident with the emergence of NS3 ± NS5A RAVs. C-SALVAGE established that a non-cross-resistant PI such as...
grazoprevir could be successfully used with a potent DAA of another class to treat patients harboring signature NS3 variants resistant to earlier PIs.

Pursuant to the endorsement of major regulatory agencies, SVR₁₂ has now become the standard endpoint for HCV treatment trials. Nonetheless, the correlation between SVR₁₂ and durable viral suppression should ideally be confirmed for each novel regimen to exclude the unlikely possibility of late relapses [9–12]. The current report presents the final follow-up week 24 results from C-SALVAGE. In addition, viruses from the relapsing patients were periodically sequenced throughout the follow-up period to explore the evolution of emergent NS3 and NS5A variants over time.

**METHODS**

**Study Design**

C-SALVAGE was an international, open-label, phase 2 trial of grazoprevir 100 mg orally once daily, elbasvir 50 mg orally once daily, and ribavirin orally twice daily at a total daily dose of 800–1400 mg based on weight for 12 weeks in patients with chronic HCV genotype 1 infection who had already failed ≥4 weeks of PR combined with boceprevir, telaprevir, or simeprevir [8]. Adults with plasma HCV RNA levels ≥10,000 IU/mL were eligible. Exclusion criteria included decompensated liver disease, hepatocellular carcinoma, human immunodeficiency virus or hepatitis B virus coinfection, thrombocytopenia <50 × 10³/µL, or hypoalbuminemia <3.0 g/dL. Patients with compensated cirrhosis were not excluded. The protocol required staging of liver fibrosis by either biopsy or noninvasive assessment within an appropriate window. All patients were to be followed at specified times for 24 weeks after cessation of study therapy; if virologic failure was diagnosed, the patient was to return approximately 2 weeks later to confirm the findings (the failure-confirmation visit). The trial was conducted in accord with the Declaration of Helsinki and Good Clinical Practice. The primary results have recently been published [8].

**Viral and Resistance Assays**

Plasma HCV RNA measurements were to be performed at post-therapy follow-up weeks 4, 8, 12, and 24, using the COBAS TaqMan version 2.0 assay (Roche Diagnostics, Branchburg, New Jersey). At baseline as well as at the time of virologic failure and thereafter, NS3 and NS5A genes were amplified using reverse transcription polymerase chain reaction, followed by population sequencing [13]. Resultant amino acid sequences were compared to wild-type HCV genotype 1a (H77) or 1b (Con1) reference sequences. Phenotypic drug susceptibilities for NS3 or NS5A variant replicons were respectively categorized as low-level (≤5-fold increase) or high-level (>5-fold increase) resistance based on the effective concentration of grazoprevir or elbasvir required to inhibit 50% of growth (EC₅₀) compared with the EC₅₀ of the subgenotype-specific wild-type reference strain [8].

**Statistical Analyses**

Success at follow-up visits was defined as HCV RNA levels below the assay limit of quantification (15 IU/mL). The primary efficacy population specified by protocol included all patients without significant protocol violations. Only observed successes or failures contributed to the primary efficacy analysis. The protocol-stipulated supportive efficacy analysis was performed on the full analysis set, which encompassed all patients who received at least 1 dose of study treatment. For this sensitivity analysis, patients with missing outcome data were counted as failures unless flanked by visits where HCV RNA levels were both <15 IU/mL. Although the primary efficacy endpoint was unquantifiable HCV RNA measured 12 weeks after the end of treatment (SVR₁₂), descriptive analyses were planned to assess response rates out to posttherapy follow-up week 24 (SVR₂₄).

**RESULTS**

**Patient Disposition**

All 79 patients (including the 30 patients with genotype 1a infections [38%] and 34 patients with cirrhosis [43%]) had been unsuccessfully treated with an NS3/4A PI in the past [8]. A total of 66 (84%) patients had a history of virologic failure. Baseline NS3 and NS5A sequencing data were available by the time of this analysis for every patient. All patients received at least 80 days of the prescribed 84-day study regimen, and 78 of 79 (99%) patients completed the full course of therapy. One patient stopped treatment 4 days early because of non-drug-related adverse events, but remained in the study for subsequent follow-up visits. Another patient who completed the prescribed course of treatment dropped out of the study at follow-up week 6 after relapsing 4 weeks after cessation of therapy. The other 78 patients (99%) completed their final scheduled visit at follow-up week 24.

**SVR₂₄ Rates**

At the end of therapy, HCV RNA was undetectable in 78 of 79 (98.7%) patients; the only subject with detectable HCV RNA below the assay limit of quantification relapsed by follow-up week 4. Overall, relapses occurred in 3 (3.8%) patients (2 with genotype 1a and 1 with genotype 1b infection) within the first 8 weeks after cessation of study therapy (2 at follow-up week 4 and 1 at follow-up week 8), yielding an SVR₁₂ rate of 96.2% (95% confidence interval [CI], 89.3%–99.2%) [8]. None of the relapses occurred in the 11 patients needing ribavirin dose reduction. In 2 of the 3 relapsing patients, the viral load quickly returned to essentially baseline levels. No further relapses occurred subsequent to follow-up week 8. Undetectable HCV RNA levels were maintained throughout the full 24 weeks of follow-up in the other 76 patients. Thus, SVR₂₄ was attained in 76 of 79 patients (96.2% [95% CI, 89.3%–99.2%]) overall, in 28 of 30 (93.3%) patients with genotype 1a infection, 63 of 66 (95.5%) patients with prior virologic failure, 33 of 36 (91.7%) patients with
<table>
<thead>
<tr>
<th>Cirrhosis Status and Type of Failure on Prior PI Treatment</th>
<th>Infected Sub-genotype and Type of Study Failure</th>
<th>HCV Parameters</th>
<th>Week 4 Follow-up</th>
<th>Week 8 Follow-up</th>
<th>Week 12 Follow-up</th>
<th>Week 24 Follow-up</th>
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<tr>
<td>Patient 1: Noncirrhotic/relapsed after PR + BOC</td>
<td>GT-1a Relapse</td>
<td>HCV RNA, IU/mL</td>
<td>1.756.431</td>
<td>1.179.252</td>
<td>1.171.094</td>
<td>Lost to follow-up after virologic failure confirmation visit at week 6</td>
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<td>NS3 variants with ≤5x GZR resistance</td>
<td>V36L, R155K</td>
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<td>Patient 2: Cirrhotic/broke through PR + TLP after TLP stoppeda</td>
<td>GT-1a Relapse</td>
<td>HCV RNA, IU/mL</td>
<td>1.756.431</td>
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<td>Lost to follow-up after virologic failure confirmation visit at week 6</td>
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<td>Patient 3: Cirrhotic/broke through PR + TLP after TLP stopped</td>
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<td>HCV RNA, IU/mL</td>
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<td>NS5A variants with &gt;5x EBR resistance</td>
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Only population sequencing was performed on follow-up specimens. Fold-change refers to the effective concentration of grazoprevir or elbasvir required to inhibit 50% of growth (EC50) of the variant replicon relative to the EC50 of the wild-type control referent. NS3A substitutions considered as signature NS3 RAVs for the older protease inhibitors: V36A/G/L/I/M, T54A/C/G/S, V55I, Y56H, Q80K/R, V107I, 122A/G/R, I132V, R156X, A156T/A/F/G, V158I, D168X, L170A/F/T/V, and M175L. NS5A substitutions considered as signature NS5A RAVs: C28T/A, Q30E/H/R/G, L31M/V/F, H58D, and 93C/H/N/S for genotype 1a and L28T/V/A, R30E/H/G/R, L31M/V/F, P58D, and 93C/H/N/S for genotype 1b. Abbreviations: @, no such variants detected; BOC, boceprevir; EBR, elbasvir; GT, genotype; GZR, grazoprevir; HCV, hepatitis C virus; ND, not done; PI, protease inhibitor; PR, pegylated interferon and ribavirin; RAV, resistance-associated variants; TLP, telaprevir; TND, target not detected.
a Patient 2 had failed a regimen of faldaprevir/PR before receiving telaprevir/PR, and therefore was excluded from the per-protocol population. HCV RNA was detectable but unquantifiable [TD(u)] in this patient at the end of study therapy.
b Viral load and sequencing results tabulated here were from specimens obtained from this patient at the failure-confirmation visit at follow-up week 6 (not week 8), after which the patient discontinued the study and was lost to follow-up.
baseline NS3 and/or NS5 RAVs, and 32 of 34 (94.1%) patients with cirrhosis. After removal of 9 patients for serious protocol violations during the treatment period, 68 of the 70 (97.1%) patients remaining in the primary per-protocol population achieved SVR24.

Time Course of Baseline and Emergent Variants
All 3 relapsing patients in C-SALVAGE had a history of virologic failure on their previous PI-based regimen. NS3 variants were detected at baseline in 3 of 3 patients (100%) who eventually relapsed and in 31 of 76 (41%) patients who achieved SVR24. Baseline NS5A variants were detected in 2 of 3 (67%) relapsing patients and in 6 of 76 (8%) patients who achieved SVR24. Six patients harbored virus with both NS3 and NS5A polymorphisms at baseline, of whom 2 relapsed and 4 achieved SVR24 (67%). Of the 4 patients with high-level grazoprevir-resistant virus at baseline, 1 relapsed and 3 achieved SVR24 (75%). Of the 5 patients with high-level elbasvir-resistant virus at baseline, 2 relapsed and 3 patients achieved SVR24 (60%). All baseline variants reappeared at the time of relapse and persisted throughout the available follow-up period.

NS3_A156T emerged in all 3 patients at relapse, followed by its rapid disappearance in 2 patients between the time of failure and the failure confirmation visit approximately 2 weeks later. For patient 1 with undetectable HCV RNA at the end of therapy, Q80K, D168N, R155T, and A156T/A were identified at follow-up week 4 (day 118); at the confirmation visit (day 135), only Q80K, D168N, and R155T were detected. For patient 2 with detectable but unquantifiable HCV RNA at the end of therapy, R155K, V36L, D168N, and A156T were identified at follow-up week 4 (day 113); at the confirmation visit (day 131), R155K, V36L, D168N, and A156T were again detected and then the patient was lost to follow-up. For patient 3 with undetectable HCV RNA at the end of therapy, T54S and A156A/T were identified at follow-up week 8 (day 141); at the confirmation visit (day 150), only T54S was detected. In contrast, there were no changes in the NS5A variants between the time of relapse and the last follow-up visit (Table 1).

DISCUSSION
In the C-SALVAGE study, 79 patients with chronic HCV genotype 1 infection who had failed earlier PI-based combination regimens were treated with grazoprevir and elbasvir plus ribavirin, including 84% with past history of virologic failure [8]. NS3 RAVs for first-generation PIs were present in almost half the patients at baseline, but high-level cross-resistance to grazoprevir in vitro was only seen in a small minority. Both SVR12 and SVR24 were achieved in all but 3 patients who had relapsed by or before follow-up week 8, yielding a durable response rate of 76 of 79 (96.2%), which likely reflects the cure rate. The sole patient with detectable but unquantifiable HCV RNA at the end of therapy relapsed. Baseline RAVs reappeared at the time of relapse in the 3 patients with virologic failure, suggesting that virus persisted below the level of detection while on treatment. Virus harboring NS3_A156A/T mixtures (confering >5 times increase in grazoprevir EC90 in vitro) transiently emerged in 2 patients at the time of relapse, but could no longer be detected at confirmation visits 2 weeks later. NS3_A156T variants were not identified at follow-up week 24 in these 2 patients. The patient who was lost to follow-up after relapsing with the NS3_A156T RAV without demonstrable wild-type virus still harbored the variant at last follow-up 2 weeks later. Unlike the new but transient NS3_A156T variant, all NS5A_RAVs at relapse persisted until the end of the follow-up period. Virus harboring NS5A_Y93H (confering a >5 times increase in elbasvir EC50 in vitro) emerged in 2 patients at relapse and remained detectable throughout the remainder of the 24-week follow-up period. An important limitation of these observations is the sensitivity of population sequencing, which can miss minority variants when their prevalence is <25% of the circulating viral quasispecies [14]. Furthermore, there were only 3 failures in the completed trial, so generalizations (as opposed to tentative hypotheses) are premature.

Cost-effective recommendations for baseline resistance testing in treatment-experienced patients await accumulating data as interferon-free DAA regimens become the standard of care for HCV infection. Phenotypic resistance testing may provide supplemental drug-specific information, as not all baseline variants actually confer classwide resistance. In the pivotal C-EDGE (Hepatitis C-Evaluation of Dual Grazoprevir/Elbasvir) trial of grazoprevir/elbasvir (without ribavirin) for treatment-naive patients, the uncommon NS5A_RAVs with >5-fold decreased susceptibility to elbasvir in genotype 1a infections at baseline, especially in the context of high viral loads, were associated with relapse; in contrast, baseline NS3_RAVs, irrespective of their effect on grazoprevir susceptibility, did not appear to significantly impact SVR12 rates [15]. Continued vigilance will help elucidate if/when/how specific NS3 or NS5A polymorphisms affect responses to grazoprevir/elbasvir.

The combination of grazoprevir and elbasvir with ribavirin given orally for 12 weeks offers a new therapeutic option for patients who have failed treatment with PR and an earlier PI, even in the presence of NS3 variants detectable by population sequencing conferring resistance to the first generation of PIs [8, 14, 15]. In treatment-naive patients, 12 weeks of grazoprevir/elbasvir without ribavirin produced a 95% SVR12 rate [15]; the contribution of ribavirin in treatment-experienced patients is being evaluated in a large phase 3 trial nearing completion. Unlike the transiently emergent NS3_A156T variant, NS5A_RAVs at relapse persisted for at least 24 weeks after cessation of therapy. If confirmed in larger numbers of patients, this finding could potentially have implications for the retreatment of the small number of patients who fail combination regimens containing an NS5A inhibitor.
Notes

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Author contributions. Study concept and design: M. N. R., J. W., E. B. Acquisition of data: M. B., X. F., S. C. G., E. Z., E. L., J. L. C., H. H., A. Y. M. H. Analysis and interpretation of data: M. B. Authors. Drafting of the manuscript: M. J. D., J. P., M. B., X. F., C. G., M. N. R. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: J. P. Final approval: All authors.

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Potential conflicts of interest. The funder, Merck & Co, Inc, is developing grazoprevir and elbasvir for treatment of hepatitis C virus infection. All authors have been investigators for Merck. M. B. has been a paid consultant for MSD, Gilead, Abbvie, and Janssen. S. C. G. has been a paid consultant for Abbvie, Bristol-Myers Squibb (BMS), Amgen, CVS Caremark, Gilead, Merck, and Novartis, has received grant support from Abbvie, BMS, Gilead, GlaxoSmithKline, Intercept, Merck, and Vertex, and has served on data monitoring boards for Tibotec/Janssen, J. L. C. has been a paid consultant for Gilead, Abbvie, Janssen, and MSD, and has received lecture fees from Gilead, Abbvie, Janssen, and MSD. H. H. has received lecture fees from Gilead, BMS, Abbvie, and MSD, and has participated on advisory boards for BMS, Gilead, and Abbvie Austria. X. F. has been a paid consultant for Gilead, Abbvie, and Janssen, and has received unrestricted grant support from Janssen. C. G., J. P., A. Y. M. H., M. J. D., M. N. R., J. W., and E. B. are employees of Merck, and own stock and/or stock options in the company. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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