Research Article

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Grazoprevir and elbasvir plus ribavirin for chronic HCV genotype-1 infection after failure of combination therapy containing a direct-acting antiviral agent

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Background & Aims: The Phase-2 C-SALVAGE study evaluated an investigational interferon-free combination of grazoprevir (a NS3/4A protease inhibitor) and elbasvir (a NS5A inhibitor) with ribavirin for patients with chronic HCV genotype-1 infection who had failed licensed DAA-containing therapy.

Methods: C-SALVAGE was an open-label study of grazoprevir 100 mg and elbasvir 50 mg QD with weight-based ribavirin BID for 12 weeks in cirrhotic and non-cirrhotic patients with chronic HCV genotype-1 infection who had not attained SVR after $\geqslant$4 weeks of peginterferon and ribavirin plus either boceprevir, telaprevir, or simeprevir. Exclusion criteria included decompensated liver disease, hepatocellular carcinoma, and HIV or HBV co-infection. The primary efficacy outcome was SVR_{12} defined as a HCV RNA level below the assay limit of quantification 12 weeks after the end of treatment.

Results: Of the 79 patients treated with $\geqslant$1 dose of study drug, 66 (84%) patients had a history of virologic failure on a regimen containing a NS3/4A protease inhibitor; 12 of the other 13 patients discontinued prior treatment because of adverse experiences. At entry, 34 (43.6%) of 78 evaluable patients harbored NS3 RAVs. SVR_{12} rates were 76/79 (96.2%) overall, including 28/30 (93.3%) patients with genotype 1a infection, 63/66 (95.5%) patients with prior virologic failure, 43/43 (100%) patients without baseline RAVs, 31/34 (91.2%) patients with baseline NS3 RAVs, 6/8 (75.0%) patients with baseline NS5A RAVs, 4/6 (66.7%) patients with both baseline NS3 and RAVs, and 32/34 (94.1%) cirrhotic patients. None of the five reported serious adverse events were considered drug-related.

Conclusions: Grazoprevir and elbasvir plus ribavirin for 12 weeks provides a promising new treatment option for patients after failure of triple therapy containing an earlier-generation protease inhibitor.

Introduction

The introduction of direct-acting antiviral agents (DAAs) set a new standard for HCV care, substantially increasing achievable rates of sustained virologic response (SVR) [1–3]. Although undoubtedly a quantum advance, a sizeable minority of patients treated with first-generation protease inhibitors combined with peginterferon alfa and ribavirin (PR) do not clear their infection. Virologic failure after DAA therapy is often accompanied by the emergence of resistance-associated variants (RAVs) which can limit subsequent treatment options [4–7]. The signature NS3 RAVs for first-generation protease inhibitors have been well characterized *in vitro*, but their full therapeutic implications remain incompletely understood [8–10]. In particular, the extent and significance of in-class cross-resistance between first and later generation protease inhibitors have not been definitively established in the clinic [10–12].

Whether patients who have not been cured by triple therapy with PR and an older protease inhibitor can be reliably salvaged with regimens incorporating a more potent protease inhibitor with a higher genetic barrier to resistance together with a DAA of another class has not been comprehensively evaluated. Earlier studies with simeprevir plus sofusbuvir indicate that SVR_{12} rates exceeding 80% might be attainable in genotype 1 infection after failure of PR plus a first-generation protease inhibitor [13]. Additional effective, well tolerated, and convenient

Keywords: C-SALVAGE; HCV genotype-1; Grazoprevir; Elbasvir; Direct-acting antiviral agents.

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Abbreviations: DAA, Direct-acting antiviral agents; PR, Peginterferon alfa and ribavirin; RAV, Resistance-associated variants; SVR, Sustained virologic response.

treatment options need to be identified for patients who are not cured by DAA ± PR combination regimens [1,3,13–18].

The C-SALVAGE study investigated the safety and efficacy of an investigational combination of grazoprevir (a NS3/4A protease inhibitor) and elbasvir (a NS5A inhibitor) with ribavirin for patients with chronic HCV genotype-1 infection who had failed licensed DAA-containing regimens. Many RAVs selected by earlier protease inhibitors remain susceptible to grazoprevir [19]. The main objective of this phase 2 trial was to explore the utility of a novel interferon-free DAA-combination in patients who had not achieved SVR after triple therapy containing a DAA in the context of emergent RAVs. Specifically, C-SALVAGE was designed to test whether a DAA-regimen anchored by a non-cross-resistant protease inhibitor could consistently clear HCV infection among patients with a history of failure on a triple regimen containing PR and a less active first-generation protease inhibitor.

Patients and methods

Study design

C-SALVAGE was an international, open-label, hypothesis-generating study of grazoprevir (100 mg PO QD), elbasvir (50 mg PO QD), and ribavirin (given PO BID at a total daily dose of 800 mg to 1400 mg based on weight) for 12 weeks in patients with chronic HCV genotype-1 infection who had failed $\geqslant$4 weeks of peginterferon and ribavirin combined with boceprevir, telaprevir, simeprevir, or sofosbuvir. Adults $\geqslant$18 years of age with plasma HCV RNA levels $\geqslant$10,000 IU/ml at screening were eligible. Exclusion criteria included decompensated liver disease, hepatocellular carcinoma, HIV or HBV co-infection, thrombocytopenia $<50 \times 10^3/\mu L$, or hypoalbuminemia <3.0 g/dl. Patients with compensated cirrhosis were not excluded but the proportion of cirrhotic patients in the study was limited to a maximum of 40%. To ensure sufficient numbers of enrolled patients with baseline NS3 RAVs, approximately 80% of the enrolled subjects were to have experienced virologic failure on prior triple therapy. Written informed consent was obtained from all participants. The trial was conducted in accord with Declaration of Helsinki and Good Clinical Practice guidelines. Subjects who discontinued treatment prior to completion were encouraged to return for all remaining study visits. Patients were to be followed for 24 weeks after the cessation of study therapy. The trial was initiated 23 May 2014 and will be ongoing until approximately 23 April 2015 when the last patient is scheduled to complete the final follow-up visit.

The protocol mandated staging of liver disease which could be accomplished by biopsy or noninvasive assessment within an appropriate timeframe. Cirrhosis was documented by a liver biopsy showing Metavir stage F4 at any time; transient elastography (Fibroscan) performed within 12 months of entry yielding a result >12.5 kPa; or biochemical markers of liver fibrosis (FibroTest or FibroSure) yielding a score of >0.75 coupled with an AST:platelet ratio index (APRI) of >2. The absence of cirrhosis could be inferred if a liver biopsy performed within the previous 24 months did not reveal cirrhosis, a Fibroscan performed within the previous 12 months had a result of $\leqslant$12.5 kPa; or a FibroSure or FibroTest score was $\leqslant$0.48 with an APRI of $\leqslant$1 in the preceding 12 months.

Viral and resistance assays

Plasma HCV RNA levels were measured by the COBAS TaqMan v2.0 assay (Roche Diagnostics, Branchburg, NJ, USA) with lower limits of quantification and detection of 15 and 9 IU/ml, respectively. Specimens for viral load measurements were to be done at screening; baseline (Day 1); treatment weeks 1, 2, 4, 6, 8, 10, and 12; and follow-up weeks 4, 8, 12, and 24 after cessation of therapy. Specimens from all participants before initiation of study therapy were used to generate baseline HCV-subtype sequence information. Additional samples were collected from patients who met the criteria for virologic failure at the time of failure and at later follow-up visits. Due to assay limitations, only samples with HCV RNA titers $\geqslant$1000 IU/ml were sequenced.

NS3 and NS5A genes were amplified using reverse transcriptase-polymerase chain reaction (RT-PCR) followed by population sequencing with a lower limit of variant detection of approximately 20–25% prevalence [19–21]. Resultant amino acid sequences were compared to wild-type HCV genotype 1a (H77) or 1b (Con1) reference sequences. Phenotypic characterization of variants was conducted using HCV replicons; resistance was characterized as low-level or high-level resistance based on the effective (inhibitory) concentration (EC_{50} $\leqslant 5 \times$ *vs.* $>5 \times$ of the wild-type referent strain, respectively).

To search for NS3 variants at baseline, all amino acid positions within NS3 protease were examined. Single NS3 amino acid substitutions involving V36A/G/L/M/I, T54A/C/G/S, V55A/I, Y56H, Q80K/R, V107I, 122A/G/R, I132V, R155X, A156S/T/V/F/G, V158I, D168X, I/V170A/F/T/V, and M175L were considered clinically relevant RAVs because these mutations had been commonly identified after treatment failures with boceprevir, telaprevir, simeprevir, or vaniprevir [12]. A subset of these first-generation protease inhibitor RAVs (involving Y56H, R155G/T/W, A156G/T/V/L, and D168A/G/T/V/L/I/F/Y/E/H/K) exhibited a >5-fold increase in grazoprevir EC_{50} in genotype 1a replicons relative to the wild-type referent [19]. Post-baseline amino acid substitutions at loci 36, 54, 55, 80, 107, 122, 132, 155, 156 158, 168, 170, and 175 were used to define emergent RAVs in virologic failures on or after study therapy.

Statistical analyses

Because C-SALVAGE was an estimation study without a control group, no formal hypothesis-testing was planned. The primary efficacy analysis prescribed by protocol estimated the proportion of patients without significant protocol violations (the per-protocol population) with a HCV RNA level below the limit of quantification (15 IU/ml) 12 weeks after the end of treatment (SVR_{12}). Only observed success or failure contributed to the primary efficacy analysis. The 95% confidence intervals for SVR rates were computed by the Clopper-Pearson method [22].

The protocol-stipulated secondary efficacy analysis and the primary safety analysis were performed on all patients who received at least one dose of study treatment (the full analysis set). For this sensitivity analysis of efficacy, patients with missing outcome data were counted as failures unless flanked by visits where HCV RNA levels were both <15 IU/ml. Adverse events occurring anytime during the treatment period and the initial 14 days of post-therapy follow-up were included in the safety analyses. Analyses based on the full data set form the focus of this report.

Exploratory analyses were performed for SVR_4 and are planned for SVR_{24}. Descriptive analyses were done for clinically relevant subgroups, such as patients with baseline RAVs and cirrhosis. SVR_{12} rates were computed by baseline NS3 RAVs categorized by their *in vitro* susceptibility to grazoprevir.

Results

Subject accounting and baseline characteristics

All 79 enrolled patients were treated with at least one dose of study drug (Fig. 1). There were 33 (42%) women, two (3%) non-whites, 34 (43%) cirrhotics (including seven diagnosed by biopsy), and 30 (38%) genotype 1a infections (Table 1). All participants had received a NS3/4A protease inhibitor; none had taken sofosbuvir. From the dates provided in the medication summaries, we estimated that the median [interquartile range] time between prior and study therapy was approximately 72 [48, 96] weeks. A total of 66 (84%) patients had a history of virologic failure. Of the remaining 13 patients with non-virologic failure, 12 had discontinued treatment because of drug intolerance or adverse events and one had received an abbreviated 12-week course of PR plus simeprevir as part of a clinical trial.

At entry, 34 (43.6%) of the 78 patients with available NS3 sequencing data harbored variants resistant to boceprevir, telaprevir, or simeprevir. Only four (11.8%) of these 34 patients with signature NS3 RAVs harbored variants with >five-fold decreased *in vitro* susceptibility to grazoprevir in a replicon assay. In addition, eight (10.1%) of the 79 patients with available NS5A sequencing data harbored virus with NS5A polymorphisms at baseline, including 5/8 (62.5%) patients with variants exhibiting >five-fold decreased susceptibility to elbasvir *in vitro*.