Review

Resistance to mericitabine, a nucleoside analogue inhibitor of HCV RNA-dependent RNA polymerase

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Mericitabine (RG7128), an orally administered prodrug of PSI-6130, is the most clinically advanced nucleoside analogue inhibitor of the RNA-dependent RNA polymerase (RdRp) of HCV. This review describes what has been learnt so far about the resistance profile of mericitabine. A serine to threonine substitution at position 282 (S282T) of the RdRp that reduces its replication capacity to approximately 15% of wild-type is the only variant that has been consistently generated in serial in vitro passage experiments. To date, no evidence of genotypic resistance to mericitabine has been detected by population or clonal sequence analysis in any baseline or on-treatment samples collected from >600 patients enrolled in Phase I/II trials of mericitabine administered as monotherapy, in combination with pegylated interferon/ribavirin, or in combination with the protease inhibitor, danoprevir, for 14 days in the proof-of-concept study of interferon-free therapy.

The approval of boceprevir and telaprevir [1,2], the first inhibitors of the non-structural (NS) 3/4A (NS3/4A) serine protease of HCV, for use in combination with pegylated interferon-α and ribavirin (triple therapy), has ushered in a new era of direct acting antiviral agents (DAAs) for the treatment of chronic hepatitis C. Triple therapy significantly increases viral eradication rates in both treatment-naive and previous non-sustained virological responders to interferon-based therapy while also decreasing the required duration of treatment for many patients [3–10]. These advances do, however, have limitations: the side-effect burden is increased and the available protease inhibitors (boceprevir and telaprevir) are only approved for treatment of HCV genotype 1 infection. In addition, these first-generation protease inhibitors have a low barrier to antiviral resistance. As a result, resistance to protease inhibitors develops rapidly in most patients when these agents are administered alone [11–13]. Resistance to DAAs appears to be characterized by outgrowth of pre-existing minor viral populations with amino acid substitutions that confer reduced susceptibility to the drug [14,15]. In combination with pegylated interferon-α and ribavirin, protease-inhibitor-resistant HCV variants are selected and grow when the interferon response is inadequate [3,4,6]. Among patients who did not achieve a sustained virological response (SVR) during clinical development trials of triple therapy, 53% of patients treated with boceprevir and 62% of patients treated with telaprevir had protease-inhibitor-resistant viral variants detected by population sequencing (or direct sequencing) as the dominant population at the time of treatment failure [16,17]. Long-term follow-up studies have shown that the wild-type viral population becomes dominant within 1–2 years in the majority of patients [18,19]. However, the resistant variants may not have cleared completely and could continue to replicate at low levels.

Mericitabine (RG7128) is an orally administered prodrug of PSI-6130 (β-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine), a potent and selective nucleoside analogue inhibitor of the NS5B protein-encoded RNA-dependent RNA polymerase (RdRp) of HCV (Figure 1). The objective of this review is to describe what has been learnt so far about the resistance profile of mericitabine, the nucleoside analogue RdRp inhibitor that is furthest advanced in terms of clinical phase development.
HCV lifecycle and DAA targets

HCV is an enveloped, single-stranded positive RNA hepatotropic virus that associates with lipoprotein particles in the circulation of infected patients. The lifecycle of HCV is shown in Figure 2. The virus gains entry into hepatocytes through a complex interaction between HCV envelope glycoproteins E1 and...

Figure 1. Structures of mericitabine® and its active metabolites, PSI-6130 and RO2433

Figure 2. Lifecycle of HCV and potential drug targets
E2 and several essential host cell surface components (glycosaminoglycans, the tetraspanin CD81, the scavenger receptor class B type-1 and the tight junction proteins claudin-1 and occludin). Entry is followed by clathrin-mediated endocytosis [20].

Viral protein synthesis and replication are cytoplasmic processes [21]. Membrane-associated HCV replication complexes generate new HCV RNA molecules while translation of RNA genomes produces a polyprotein comprising approximately 3,000 amino acids that is subsequently cleaved by host and viral proteases into 10 structural and NS proteins. Capsid proteins and genomic RNA are assembled into nucleocapsids that bud into the lumen of the endoplasmic reticulum. Newly formed virions mature in the Golgi apparatus before exiting the cell through the secretory pathway [21].

The lifecycle of HCV presents a number of potential targets against which many DAA drugs are currently being developed [15,22]. The most advanced drug development programmes to date have targeted polyprotein processing (by inhibiting the NS3/4A serine protease) and HCV replication (through different mechanisms). Two NS3/4A serine protease inhibitors (boceprevir and telaprevir) that target polyprotein processing and, as a result, HCV production have now been approved for the treatment of HCV infection in both the USA and Europe. In addition, at least 10 other molecules in this class have been and/or are being investigated in humans and at least 4 other classes of drugs that inhibit HCV replication are currently in clinical trials. These include nucleoside/nucleotide analogue inhibitors and non-nucleoside inhibitors of RdRp, inhibitors of NS5A, a viral protein involved in the regulation of HCV replication, and inhibitors of cyclophilin A, a host cell protein required for HCV replication [15,22].

**HCV resistance to DAAs**

DAAs provide not only an opportunity to improve cure rates for patients with chronic hepatitis C, but also a challenge because HCV is prone to the rapid selection of resistant variants when exposed to these drugs [15]. The large viral population and rapid replication rate of HCV, combined with the fact that the viral RdRp makes errors during replication that are not corrected in the absence of proofreading activity, implies that HCV exists as a quasispecies in any given patient, that is, a mixture of genotypically distinct but closely related viral populations [15]. Resistance to DAAs emerges when minority variants in a patient’s quasispecies are selected during treatment because they carry amino acid substitutions that modify the drug binding site and confer a survival advantage in the presence of the drug. Sustained antiviral pressure on the majority wild-type virus creates vacant replication space for the resistant variant, which can subsequently grow and fill in this space [15].

Resistance profiles vary greatly between classes of DAAs but less so between drugs in the same class. The binding site of a particular DAA is important in determining the potential for cross-resistance. Two different drugs that bind to different ‘pockets’ on the same molecule or to two entirely different proteins (‘targets’) are unlikely to show cross-resistance. By contrast, two different compounds that bind to the same pocket on the same target are likely to exhibit cross-resistance.

Viral replication is suppressed when the serum drug concentration remains above a threshold. Thus, drug exposure, which reflects the amount of drug absorbed into the blood and the concentration that is sustained during the dosing interval, is an important factor in the prevention of resistance to a DAA.

The frequency and speed at which resistance develops when a given patient is treated with a DAA depends on the genetic barrier to resistance (number of amino acid substitutions required to generate resistance to the drug), the fitness of resistant variants, the level of resistance the mutant(s) confers and exposure to the drug. Altogether, these parameters account for the so-called ‘barrier to resistance’ of the drug [15,23,24]. When resistance does develop, it generally manifests as a virological breakthrough, that is, a re-increase in the HCV RNA level. Several different breakthrough patterns associated with antiviral resistance have been characterized. For example, if a high degree of resistance is conferred by an amino acid substitution and the fitness of the virus is only modestly affected, then the escape pattern will be characterized by a rapid increase in HCV RNA level despite ongoing DAA administration. Conversely, if the degree of resistance is lower and/or the fitness of the resistant virus is impaired, the slope of the HCV RNA level rebound will be less steep [15,23,24].

If a drug is highly efficacious against wild-type virus, resistance can emerge relatively early in treatment. However, the selection and emergence of resistance-associated mutations can be much slower when treated with a DAA that is less active against wild-type virus.

Among the drug classes that are currently in human clinical trials, the NS3/4A protease inhibitors, the non-nucleoside inhibitors of HCV RdRp and the NS5A inhibitors have been reported to have a low barrier to resistance [15,23]. When telaprevir is given as monotherapy, a virological breakthrough can be observed in HCV RNA levels within days, and resistant variants are detected as the dominant viral population at the expense of the wild-type virus.
the time of breakthrough [11,25]. Patients infected with HCV subtype 1a develop telaprevir, boceprevir and danoprevir resistance more often and more rapidly than those infected with subtype 1b [16,17,26]. This is due, at least in part, to the fact that only one nucleotide change is required in subtype 1a, but two in subtype 1b, to generate the key amino acid change at position 155 of the NS3/4A protease that confers resistance to the drugs [27]. HCV that is resistant to telaprevir is extensively cross-resistant to boceprevir and to all of the other first-generation NS3/4A protease inhibitors in development, but not to drugs from other classes [15,23,28].

Non-nucleoside inhibitors of RdRp have been developed that bind to one of four allosteric sites of the viral polymerase [15]. Available data suggest that drugs in this class have a low barrier to resistance, with extensive cross-resistance among drugs targeting the same site. Cross-resistance may also occur between drugs targeting different allosteric sites, albeit not frequently; however, there is no cross-resistance with drugs from other classes [15]. When the non-nucleoside inhibitor of RdRp filibuvir was given as monotherapy for 8–10 days, breakthrough occurred in approximately 50% of patients [29]. Mutations at NS5B position 423 were consistently associated with breakthrough [29].

NS5A inhibitors also have a low genetic barrier to resistance. They are able to select resistant variants with substitutions in the NS5A region, with no cross-resistance with drugs from other classes [15]. The combination of two drugs with a low barrier to resistance did not appear to substantially increase the overall barrier to resistance in recent trials. For example, during a study of daclatasvir (BMS-790052), an NS5A inhibitor, and asunaprevir (BMS-650032), an NS3/4A protease inhibitor, 4 of 11 patients experienced SVR, an important proof of concept, but variants resistant to both drugs were detected in all six patients who experienced breakthrough [30]. Variants in the NS5A domain included Q30E/R, L31M/V and Y93C/N, and those in the NS3 domain included R155K and D168A/E/T/V/Y [30]. Similarly, breakthrough during treatment with a non-nucleoside polymerase inhibitor (GS-9190) and a protease inhibitor (GS-9256) was associated with emergence of resistance mutations in both the polymerase (Y448H) and NS3 (R155K and/or D168V/E/N) domains [31].

The remaining two classes of drugs, namely cyclophilin inhibitors and nucleoside/nucleotide analogues, have been associated with more favourable resistance profiles when given as monotherapy. Cyclophilin inhibitors target a host protein that plays a key role in viral replication by interacting with viral proteins in the replication complex, although the molecular mechanisms are still unknown. Low-level in vitro resistance to the most advanced cyclophilin inhibitor, alisporivir, is conferred by amino acid substitutions in the NS2 and, more commonly, in the NS5A coding regions. Little is known of the potential for clinically meaningful resistance in patients with chronic hepatitis C receiving this drug; however, in vivo the variants appear to be poorly fit and they are rarely selected [15,32,33].

Several nucleoside/nucleotide analogue inhibitors of HCV RdRp have been evaluated in clinical trials. Drugs in this class generally have a high barrier to resistance due to poor in vivo fitness of selected variants. Cross-resistance is possible between agents with a common binding site or mechanism of action. For example, 2'-methyl substituted nucleosides such as PSI-6130, the active derivative of mericitabine, and PSI-7977 select for substitutions at amino acid position 282. By contrast, R1479 (4'-azidocytidine; the active drug released by hydrolysis of the produg balapiravir, which is no longer in development) selects for amino acid substitutions at amino acid position 96 and 96+142 and there is no cross-resistance in vitro between this drug and the 2'-methyl substituted nucleosides [34]. Similarly, significant resistance to PSI-938 requires the presence of three mutations (S15G, C223H and V321I) [35]; however, this purine analogue inhibitor of RdRp is active against S282T variants [36].

Mode of action and in vitro potency of mericitabine

The target for mericitabine is the catalytic site of the HCV RdRp. Several steps are necessary to activate its derivative PSI-6130. After oral administration, mericitabine (the prodrug) is hydrolysed to PSI-6130. Next, after being taken up by hepatocytes, PSI-6130 is converted to two pharmacologically active triphosphate metabolites [37–39]. The 5'-monophosphate derivative (PSI-6130-MP) undergoes conversion to diphosphate and triphosphate metabolites by cellular kinases. PSI-6130-MP also undergoes deamination to form a uridine metabolite (RO2433) that is subsequently phosphorylated (Figure 1) [39]. In vitro experiments in primary human hepatocytes have shown that PSI-6130-TP and RO2433-TP are the predominant intracellular metabolites, and that steady-state levels are reached within 48 h of exposure to the parent compound in this model [37]. The triphosphorylated moiety serves as alternative substrates that compete with natural cytidine-triphosphate at the active site of the RdRp for incorporation into the nascent viral RNA. This results in chain termination, which is thought to occur through
Steric hindrance between the bulky 2′C-methyl groups of PSI-6130-TP and RO2433-TP and the ribose moiety on the next incoming nucleotide (Figure 3) [37–39].

Mericitabine is active against all HCV genotypes (1–6) [40], but has been most extensively studied against HCV genotype 1. PSI-6130-TP and RO2433-TP have similar potency for inhibition of RNA synthesis, with 50% inhibitory concentration (IC₅₀) values of 0.34 and 1.19 µM, respectively, for the native HCV replicase complex isolated from replicon cell lines, and 0.13 and 0.52 µM, respectively, for the recombinant RdRp in a cell-free enzyme assay [37]. PSI-6130-TP has equal inhibitory potency in both subtype 1a and 1b. In vitro in a transient replicon system, PSI-6130 was equipotent against genotype 1a and 1b, with 50% effective concentration (EC₅₀) values ranging from 0.6 to 1.41 µM for different subtype 1b clinical isolates (0.51 µM for the reference strain Con1), and from 0.20 to 0.43 µM for different subtype 1a isolates (0.30 µM for the reference strain H77) [41].

Mechanisms of resistance to mericitabine and preclinical data

Several nucleoside analogue inhibitors of HCV RdRp that have 2′-C-methyl substituents including 2′-C-methyladenosine, 2′-C-methylcytidine (NM107; a derivative of valopicitabine, which was discontinued due to toxicity) and 2′-C-methyl-7′-deaza-adenosine (MK-0608; another discontinued compound), select for a common amino acid substitution, a change from serine to threonine at position 282 (S282T), in the HCV replicon system [42–44]. This substitution is located in the active site of HCV RdRp and results in an 11-fold reduction in the catalytic efficiency of the enzyme compared with wild-type [42]. In the genotype 1b replicon system, the replication capacity of S282T-containing RdRp was approximately 15% of the wild-type enzyme [41].

Selection of replicons resistant to PSI-6130 required approximately 6 months of sequential passages in cell culture with exposure to increasing concentrations of the drug [41]. Throughout the three independent experiments, the initial concentration of PSI-6130 was 2.5 µM (approximately 5× the EC₅₀) and was ultimately increased to a concentration of 100 µM at passage 53. As cells were passaged with increasing concentrations of the drug, increasing numbers of amino acid substitutions were detected. However, the S282T substitution was the only one that emerged during all three sets of experiments, and it emerged only after 20 or more cell passages and exposure to a PSI-6130 concentration of at least 30 µM in each experiment [41]. Of the other amino acid substitutions that emerged with S282T during these experiments, none appeared consistently in all three experiments.

The presence of the S282T substitution reduces the susceptibility of the HCV RdRp to different 2′-C-methyl-containing analogues [42]; however, the extent of the reduction varies with the particular compound. For example, S282T decreases the

![Crystal structure of RdRp active site with PSI-6130 modelled](image)

Residue S282T is depicted.

Table 1. In vitro resistance conferred by the S282T substitution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Metabolite</th>
<th>Mean EC₅₀</th>
<th>Wild-type, µM</th>
<th>S282T, µM</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active metabolites of mericitabine</td>
<td>PSI-6130-TP</td>
<td>0.13</td>
<td>0.70</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RO2433-TP</td>
<td>0.52</td>
<td>1.0</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>Active metabolite of valopicitabine</td>
<td>NM107-TP</td>
<td>0.09</td>
<td>1.0</td>
<td>111.1</td>
<td></td>
</tr>
</tbody>
</table>

Comparative in vitro efficacy of the active metabolites of mericitabine and valopicitabine in wild-type versus S282T-containing recombinant HCV RNA-dependent RNA polymerase in a cell-free enzyme assay [41]. EC₅₀, 50% effective concentration.
susceptibility of RdRp to PSI-6130-TP by approximately 5-fold and to NM107-TP by approximately 111-fold in vitro (Table 1) [41]. The lesser effect of the S282T substitution on PSI-6130 than NM107 and other 2'-C-substituted nucleoside analogues may be due to the presence of the fluorine substituent, which may increase the structural flexibility of the molecule in the active site of RdRp and reduce the extent of steric hindrance [41]. By contrast, PSI-938 and PSI-661, prodrugs of β-d-2’-deoxy-2’-α-fluoro-2’-β-C-methylguanosine-5’-monophosphate show no loss of activity against replicons with the S282T substitution, although they also possess a 2’-C-methyl moiety.

The experimental introduction of the S282T amino acid substitution into a wild-type RdRp resulted in a 3.5–8-fold decrease in mericitabine susceptibility in the replicon system (using a laboratory reference strain and genetically different clinical isolates as backbones) and in an RdRp enzyme assay (using a laboratory reference enzyme) [41].

Other amino acid substitutions that emerged and persisted after exposure to PSI-6130 in in vitro experiments include K81R, K72M, I239V, I239L, L320F, A396G, A421V, C575S and Y586C. Similar to the S282T variant, these substitutions generally reduced the replication capacity of a genotype 1b replicon compared with wild-type [41]. Moreover, the reduction in replication capacity was magnified when these mutations were combined with S282T. When present alone, none of these mutations altered the susceptibility of the replicon to PSI-6130, mericitabine or NM107. However, the combination of S282T plus C575S and S282T plus L320F produced further reductions in the susceptibility of replicons to PSI-6130 beyond that seen with S282T alone. By contrast, the introduction of I239L, A396G or A421V to a replicon containing S282T appeared to partially compensate for the loss of susceptibility conferred by S282T [41].

Collectively, these observations from in vitro studies demonstrate that resistance to the active metabolite of mericitabine results from the selection of HCV variants bearing one particular amino acid substitution (S282T). However, this variant is difficult to select in vitro and S282T significantly impairs the replication capacity of the resulting HCV strain. Moreover, S282T-containing variants have slightly higher susceptibility to PSI-6130-TP than to NM107-TP, the active metabolite of valopicitabine. Only when S282T is accompanied by accessory mutations, such as C575S or L320F, is a further reduction in susceptibility to mericitabine observed. Cross-resistance between mericitabine and members of other DAA classes has not yet been observed [41,44].

**Resistance to mericitabine in clinical trials**

Mericitabine is currently in Phase II clinical trials. To date, efficacy and safety data are available from studies in treatment-naive and treatment-experienced patients, including individuals infected with HCV genotypes 1, 2 and 4. Mericitabine has been evaluated as monotherapy [45], in combination with pegylated interferon plus ribavirin up to 24 weeks [46–49] and in combination with the HCV protease inhibitor danoprevir in the proof-of-concept study of a dual oral interferon-free DAA regimen for 2 weeks [50].

Collectively these trials have enrolled >600 patients. All patients enrolled in the trials have been monitored for the development of resistance to mericitabine. Consistent definitions of clinical responses were used to identify patients for resistance monitoring during these trials and population and clonal sequence analysis techniques were used to analyse samples in resistance studies (Table 2). There was one case of breakthrough during combination therapy with pegylated interferon and ribavirin and five patients had an HCV RNA level >1,000 IU/ml after 4 weeks of therapy with pegylated interferon and ribavirin [51]. Only one patient experienced a breakthrough during dual combination therapy with danoprevir in the INFORM-I trial [50]. Analyses of samples from these patients have
not detected any evidence of selection of HCV variants resistant to mericitabine thus far. Importantly, S282T has not been detected in any clinical samples by population or clonal sequence analysis, or by ultra-deep sequencing to date [50,52–54]. Similar findings demonstrating that resistance mutations at residue S282 are uncommon at baseline have been reported in several other studies of large multinational cohorts of treatment-naive patients with either genotype 1/3 HCV monoinfection or HCV–HIV coinfection [55–57]. By contrast, variants resistant to other DAAs (protease inhibitors and non-nucleoside polymerase inhibitors) have been detected in some baseline samples from patients in trials evaluating mericitabine. Resistance to mericitabine from a long-term, Phase II trial of interferon-free treatment with mericitabine and danoprevir (INFORM-SVR) are awaited.

Short-term Phase I trials of mericitabine alone or in combination with pegylated interferon–α2a plus ribavirin

Mericitabine was first evaluated as monotherapy in a Phase I 14-day multiple ascending-dose trial in 32 patients infected with HCV genotype 1 who had not responded to previous interferon-based treatment. At dosages ranging from 750 to 3,000 mg/day, mericitabine produced dose-dependent reductions in HCV RNA levels. The greatest mean reduction in HCV RNA (2.7 log₁₀ IU/ml, range 1.2–4.1 log₁₀ IU/ml) was achieved at day 15 with the highest dosage level (1,500 mg twice daily), although even at lower doses mean changes from baseline at day 15 were 0.9 log₁₀ IU/ml (750 mg once daily, range 0.67–1.10 log₁₀ IU/ml) and 1.48 log₁₀ IU/ml (1,500 mg once daily, range 0.9–2.5 log₁₀ IU/ml) [45]. HCV RNA levels were unchanged after 14 days in eight patients who received oral placebo in this study.

Next the drug was evaluated in short-term (4-week) randomized placebo-controlled trials in combination with pegylated interferon–α2a (40 KD) and ribavirin. The first trial enrolled treatment-naive patients with HCV genotype 1 infection who were randomized to mericitabine-based triple therapy (n=65) or pegylated interferon–α2a plus ribavirin (n=16) [47]. A rapid virological response (RVR), defined as an HCV RNA level <15 IU/ml at week 4, was achieved in 88% and 85% patients, respectively, who received oral mericitabine at a dosage of 1,000 mg twice daily or 1,500 mg twice daily in combination with pegylated interferon–α2a plus ribavirin [47]. By contrast, the RVR rate was 19% in patients randomized to the pegylated interferon–α2a/ribavirin control group. Factors significantly associated with the change in HCV RNA level at week 4 in this trial included treatment with mericitabine, baseline HCV RNA level and race/ethnicity.

By contrast, patient weight, gender and HCV subtype were not significantly associated with the decrease in HCV RNA level in patients randomized to mericitabine [47].

The second trial included patients infected with genotypes 2 or 3 who had experienced non-response or relapse to a previous course of interferon-based therapy. A total of 20 patients were randomized to mericitabine-based triple therapy and five to a pegylated interferon–α2a/ribavirin control group [47]. Of them, 90% achieved an RVR when re-treated with mericitabine 1,500 mg twice daily in combination with pegylated interferon–α2a plus ribavirin [46].

Resistance in Phase I monotherapy and combination therapy trials

The collective results of resistance studies conducted in patients enrolled in the Phase I monotherapy and combination studies were recently reported [53]. The S282T variant was not detected by population sequence analysis in baseline samples collected from any of the 145 patients enrolled in these trials. Of 117 patients who received mericitabine in these studies, a total of 13 individuals met the criteria for resistance monitoring, including 5 who experienced a viral breakthrough (defined as an increase of 0.5 log in HCV RNA from nadir) and 8 who had a partial response (Figure 4 and Table 3). None met the criteria for non-response. The S282T variant was not detected in any on-treatment samples from these 13 patients. It was also not detected by clonal sequence analysis of 1,165 molecular clones from baseline isolates and 1,250 molecular clones from on-treatment isolates from the patients who met the criteria for resistance monitoring (approximately 90 clones/sample were sequenced) [53]. In longer-term Phase II trials, mericitabine was administered to patients infected with HCV genotypes 1 or 4 at a dosage of 500 mg twice daily or 1,000 mg twice...
daily for 8 or 12 weeks (PROPEL; n=408) or at a dosage of 1,000 mg twice daily for 24 weeks (JUMP-C; n=166) in combination with pegylated interferon-α2a plus ribavirin.

The results of a planned week 12 interim analysis of PROPEL showed that the complete early virological response rate (cEVR; HCV RNA level <15 IU/ml at week 12) ranged from 80% to 88% among patients randomized to receive mericitabine-based triple therapy for 12 weeks (n=324). The cEVR rate was 68% in patients who had received 8 weeks of mericitabine-based therapy and 49% in those in the control group (pegylated interferon-α2a plus ribavirin; n=84) [48].

Non-response (defined as <0.5 log decrease in HCV RNA level from baseline after 2 weeks of treatment) or virological breakthrough (defined as ≥1 log increase in HCV RNA level above the nadir) was not observed in any patient during treatment with mericitabine for up to 12 weeks in PROPEL. Moreover, no evidence of S282T was detected by population or clonal sequencing (approximately 90 clones/sample) of selected baseline or on-treatment samples [58]. A total of 11 patients were classified as partial responders because they had an HCV RNA level >1,000 IU/ml at end of mericitabine treatment. Amino acid changes observed in on-treatment samples collected from the 11 patients with a partial response were not consistent between patients enrolled in the trial or with changes observed in other trials of mericitabine. Phenotypic drug susceptibility data showed that the samples were equally susceptible before and at the end of mericitabine treatment, suggesting that these variants do not play a role in the partial responses observed in these individuals [58].

The randomized, double-blind, placebo-controlled JUMP-C study is evaluating response-guided therapy with a mericitabine-based triple therapy regimen in patients infected with HCV genotypes 1 or 4 [49]. For mericitabine recipients who remained HCV-RNA-negative between weeks 4 and 22 (extended RVR [eRVR]), all treatment was stopped at week 24, whereas those individuals who did not achieve an eRVR stopped mericitabine at week 24 but continued pegylated interferon-α2a plus ribavirin to complete 48 weeks of treatment. The control group comprises patients randomized to 48 weeks of pegylated...
Mericitabine resistance profile

Table 3. Patients selected for viral resistance monitoring in mericitabine studies

<table>
<thead>
<tr>
<th>Patient (HCV genotype)</th>
<th>Mericitabine dose</th>
<th>Reason for resistance monitoring</th>
<th>Sample day</th>
<th>Amino acid changes detected in RdRp compared with baseline sequences from the same patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mericitabine monotherapy (n=32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (1a)</td>
<td>750 mg once daily</td>
<td>Breakthrough</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>B (1a)</td>
<td>1,500 mg once daily</td>
<td>Breakthrough</td>
<td>14</td>
<td>M423I</td>
</tr>
<tr>
<td>C (1a)</td>
<td>1,500 mg once daily</td>
<td>Breakthrough</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>D (1b)</td>
<td>1,500 mg twice daily</td>
<td>Partial response</td>
<td>14</td>
<td>F162F/Y, S218A/S, K254K/R, T267I/T, A/V400A, A442A/T, I585I/V</td>
</tr>
<tr>
<td>E (1b)</td>
<td>1,500 mg twice daily</td>
<td>Partial response</td>
<td>14</td>
<td>K/M426M, H/Y452Y</td>
</tr>
<tr>
<td>Mericitabine plus pegylated interferon-α2a (40 KD) plus ribavirin combination therapy (n=85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (1a)</td>
<td>1,500 mg twice daily</td>
<td>Breakthrough</td>
<td>28–29</td>
<td>I/V11V, A/S15A, A73A/V, C/S110S, K/Q206Q, Q/R300R, D444A</td>
</tr>
<tr>
<td>G (3a)</td>
<td>1,500 mg twice daily</td>
<td>Breakthrough</td>
<td>19</td>
<td>I432V</td>
</tr>
<tr>
<td>H (3a)</td>
<td>1,500 mg twice daily</td>
<td>Breakthrough</td>
<td>21</td>
<td>A/V67V, V147M, A/V150A, I/T184I, S376N</td>
</tr>
<tr>
<td>J (1a)</td>
<td>500 twice daily</td>
<td>Partial response</td>
<td>29</td>
<td>N/S130S, F/Y162F, S180G, S189N/S, N/S231S, K/R270R, A/V421A</td>
</tr>
<tr>
<td>K (1a)</td>
<td>500 twice daily</td>
<td>Partial response</td>
<td>29</td>
<td>K510R, F/L572L</td>
</tr>
<tr>
<td>L (1a)</td>
<td>500 twice daily</td>
<td>Partial response</td>
<td>29</td>
<td>E/K77K, C/S110S, A/V178V, S556G</td>
</tr>
<tr>
<td>M (1a)</td>
<td>500 twice daily</td>
<td>Partial response</td>
<td>29</td>
<td>S470G/S, K523K/R, S543G/S, A553A/T</td>
</tr>
</tbody>
</table>

Amino acid changes relative to baseline identified by population sequencing in patients selected for resistance monitoring in clinical studies of mericitabine monotherapy and triple mericitabine plus pegylated interferon-α2a plus ribavirin combination therapy [53]. Breakthrough, increase in HCV RNA level ≥0.5 log10 IU/ml above nadir before the end of therapy; partial response, initial reduction in HCV RNA level after initiation of therapy followed by plateau; RdRp, RNA-dependent RNA polymerase.

Table 4. Patients selected for resistance monitoring on the JUMP-C trial

<table>
<thead>
<tr>
<th>Patients</th>
<th>Patient group</th>
<th>S282T variants detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline sequence analysis (all patients)</td>
<td>Genotype 1a (n=119)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Genotype 1b (n=41)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Genotype 4 (n=3)</td>
<td>None</td>
</tr>
<tr>
<td>Virological response (HCV RNA&lt;15 IU/ml; n=80)</td>
<td>–</td>
<td>No samples tested (no breakthrough)</td>
</tr>
<tr>
<td>Partial response (HCV RNA=2,000 IU/ml; n=1)</td>
<td>–</td>
<td>None</td>
</tr>
<tr>
<td>Patients with an eRVR</td>
<td>SVR-12 (n=37)</td>
<td>No samples tested</td>
</tr>
<tr>
<td></td>
<td>Relapse (n=10)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Failure to return (n=2)</td>
<td>No samples tested</td>
</tr>
</tbody>
</table>

Patients selected for resistance monitoring and results of testing for mericitabine-resistant variants in the JUMP-C study [49]. eRVR, extended rapid virological response defined as HCV RNA<15 IU/ml at every time point between weeks 4 and 22.

interferon-α2a plus ribavirin (n=85). A planned interim analysis at week 36 showed that 60% (49/81) of patients randomized to mericitabine-based triple therapy achieved an eRVR; of these, 76% (37/49) achieved an SVR-12 (HCV RNA<15 IU/ml) at week 12 of untreated follow-up [49]. The SVR rates in patients with an eRVR were similar irrespective of IL28B genotype.
A summary of the resistance analysis from JUMP-C is shown in Table 4. Among mericitabine-treated patients, all but one individual had undetectable HCV RNA levels (<15 IU/ml) by week 24 and none experienced viral breakthrough. The patient with the partial response had an HCV RNA level of approximately 2,000 IU/ml at the end of triple therapy (week 24) and no amino acid substitutions associated with resistance have been identified to date in any sample collected from this individual. Among patients included in the interim analysis who have completed treatment and follow-up, 10 (24%) individuals experienced a virological relapse, none of whom had detectable variants with substitutions associated with mericitabine resistance.

Presently, neither the final SVR rates nor the results of on-treatment resistance analyses have been reported for patients assigned to 48 weeks of treatment in JUMP-C.

Mericitabine plus danoprevir dual oral interferon-free combination regimen
Two weeks of treatment with various dosage regimens of mericitabine and the NS3/4A protease inhibitor danoprevir produced median reductions in serum HCV RNA levels of 3.7–5.2 log_{10} IU/ml from baseline in the proof-of-concept study of a dual oral interferon-free regimen in patients with chronic hepatitis C genotype 1 (INFORM-1) [50]. Only 1 of 73 patients dosed in this study experienced viral breakthrough, however, no evidence of the S282T variant nor of resistance variants typically associated with protease inhibitor treatment were detected by population or clonal sequencing (80 to >100 clones assessed per sample) in this patient.

A danoprevir-resistant variant (D168E) was detected in the baseline sample collected from one patient assigned to mericitabine 1,000 mg twice daily and danoprevir 200 mg every 8 h. When this sample was tested in vitro, susceptibility to danoprevir was shown to be reduced by approximately 37-fold, but susceptibility to mericitabine remained unaltered compared with wild-type. The patient experienced a continuous decline in serum HCV RNA of 2.7 log_{10} IU/ml during dual treatment [50].

The combination of mericitabine and danoprevir is currently being evaluated in two ongoing trials. In the INFORM-SVR trial, interferon-naive patients with HCV genotype 1 infection are receiving mericitabine (1,000 mg twice daily) and ritonavir-boosted danoprevir (100/100 mg twice daily) with or without ribavirin [59]. The duration of treatment in the trial is based on the time to become HCV-RNA-negative.

The MATTERHORN trial is evaluating mericitabine (1,000 mg twice daily) in combination with ritonavir-boosted danoprevir (100/100 mg twice daily) in HCV genotype 1 patients with a previous partial response or null response to pegylated interferon plus ribavirin [60]. MATTERHORN will determine the efficacy of a triple oral combination regimen (mericitabine, danoprevir/ritonavir and ribavirin), a protease-inhibitor-based triple therapy combination regimen (danoprevir/ritonavir, pegylated interferon-α2a and ribavirin) and a quadruple therapy regimen (mericitabine, danoprevir/ritonavir, pegylated interferon-α2a and ribavirin). All regimens are administered for 24 weeks in the study, with the exception of one treatment group of previous non-responders who are receiving 24 weeks of quadruple therapy followed by 24 weeks of pegylated interferon-α2a plus ribavirin (total duration of 48 weeks). Formal analyses of resistance to mericitabine and danoprevir will be reported in this trial.

Conclusions
The approval of the first DAAs for treatment of chronic hepatitis C will stimulate further research to identify combination regimens that maximize viral eradication rates, shorten the duration of treatment and reduce the side-effect burden. Highly potent agents with a broader spectrum of activity and different resistance profiles will be required in the near future. Drugs with a high barrier to resistance like nucleoside/nucleotide analogues may be particularly useful in this context.

Mericitabine is a prodrug of the potent nucleoside analogue inhibitor of RdRp PSI-6130 that has a demonstrated high barrier to resistance both in vitro and in patients with chronic hepatitis C. To date, there has been no evidence of the in-vitro-identified mericitabine-resistant variant S282T at baseline and up to 24 weeks of triple therapy with mericitabine, pegylated interferon-α2a and ribavirin in clinical trials. This variant has not been selected when mericitabine was used for 2 weeks in combination with danoprevir, an HCV protease inhibitor, in an interferon-free regimen [48,50]. Although restricted to short-term administration, the absence of resistance to either drug after 2 weeks of combination therapy with mericitabine plus the protease inhibitor danoprevir suggests that mericitabine has a protective effect against resistance to danoprevir (that is typically observed after 7 days of monotherapy) [61].

Random variants with other amino acid substitutions have been observed to arise sporadically during and after treatment with mericitabine; however, these variants have not been associated with diminished susceptibility to the drug. Pending the results from ongoing clinical trials with mericitabine in
interferon-free regimens [59,60], it can be concluded that mericitabine has a high barrier to resistance in combination with pegylated interferon and ribavirin and that the selection of resistant variants was not observed. The findings thus far underscore the potential for mericitabine and other nucleoside or nucleotide analogues to help avoid the emergence of resistance seen in trials with drugs that have a lower barrier to resistance. Reinforcement of the emerging impression that mericitabine has a high barrier to resistance will require monitoring for resistant variants in ongoing and future clinical trials including those investigating interferon-free regimens.

Acknowledgements

All authors have contributed to the work and writing of the manuscript and they have all read and approved the paper. They take full responsibility for its scientific content. Support for third-party writing assistance for a preliminary draft of this manuscript, furnished by Blair Jarvis, was provided by F Hoffmann-La Roche Ltd.

Disclosure statement

J-MP has received research grants from Gilead and Roche. He has served as an advisor for Abbott Laboratories, Anadys, Biotica, Boehringer-Ingelheim, Bristol-Myers Squibb, DebioPharm, Gilead, GlaxoSmithKline, Idexix, Janssen-Cilag, Madaus-Rottapharma, Schering-Plough/Merck, Novartis, Pfizer, Pharmasset, Roche, Vertex and Virco. IN is an employee of Hoffmann-La Roche (Nutley, NJ, USA). IJ has acted as a consultant to Abbott Laboratories, Achillion, Biolex, Boehringer, Roche/Genentech, Tibotec, Bristol-Myers Squibb, Novartis, Gilead, Pfizer, Vertex, Schering Plough/Merck, Boehringer-Ingelheim, Pharmasset; has received research support from Schering Plough/Merck, Gilead, Novartis, Boehringer-Ingelheim, Pharmasset, Roche/Genentech, Tibotec/Janssen, Anadys, GlobImmune; and acted as a speaker for Schering-Plough/Merck, Gilead, Bristol-Myers Squibb, Genentech and Vertex.

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