

# Treating HCV with ribavirin analogues and ribavirin-like molecules

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**Nucleos(t)ide analogues have proven useful in the treatment of viral infections. Ribavirin is a nucleoside, guanosine analogue, whose mechanisms of action include inhibition of inosine monophosphate dehydrogenase (IMPDH), which is the key step in *de novo* guanine synthesis, a requirement for viral replication. In combination with pegylated interferon alfa, ribavirin is the standard of care for the treatment of chronic hepatitis C today. However, the medication is associated with significant haemolytic anaemia, which may require dose reduction, discontinuation or treatment with recombinant human erythropoietin. Dose reduction also appears to decrease sustained viral clearance rates. Newer IMPDH inhibitors are in various stages of development. Viramidine, a liver-targeting prodrug of ribavirin, has demonstrated significant antiviral activity and erythrocyte-sparing properties. It is currently in Phase 3 trials. Clinical trials of merimepodib, another investigational IMPDH inhibitor, have completed enrolment for a Phase 2b study as a third medication for administration with pegylated interferon plus ribavirin. Although other IMPDH inhibitors also have antiviral activity, these medications appear best suited as immunosuppressive medications at this time.**

Keywords: inosine monophosphate dehydrogenase, merimepodib, viramidine, anaemia, antiviral treatments

## Introduction

Clinical studies of combination therapies for chronic hepatitis C have shown sustained viral response (SVR) rates of ~55%.<sup>1,2</sup> Efforts to raise this rate of response have been blocked by numerous obstacles involving patient, viral and medication factors, including patient- and virus-related variables (e.g. cirrhosis, viral levels, race, genotype), adverse events requiring dose reductions or medication discontinuation (e.g. anaemia, depression, cytopenias) and non-adherence to treatment regimens. For example, an essential component of therapy for chronic hepatitis C is ribavirin, a nucleoside analogue that inhibits inosine monophosphate dehydrogenase (IMPDH), among other mechanisms of action; however, ribavirin is also associated with potential dose-limiting haemolytic anaemia that compromises SVR rates.<sup>3,4</sup> Pharmaceutical manufacturers are responding by developing a number of new, safer anti-hepatitis C virus (HCV) medications including IMPDH inhibitors, protease inhibitors, polymerase inhibitors, helicase inhibitors, internal ribosomal entry site inhibitors, small and expressed interfering RNAs, ribozymes and several new interferons (e.g. albumin-interferon alfa, consensus-interferon, interferon- $\gamma$ ).<sup>5,6</sup> Several ribavirin-like molecules presently under development have the potential to improve outcome compared with standard ribavirin.

## Ribavirin

Ribavirin, first discovered in 1970, is a guanosine analogue with broad-spectrum antiviral activity.<sup>7,8</sup> The medication's biochemical

name is 1- $\beta$ -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.<sup>7</sup> It has a chemical formula of C<sub>8</sub>H<sub>12</sub>N<sub>4</sub>O<sub>5</sub> and a molecular weight of 244.2 (Figure 1).<sup>7</sup>

## Pharmacology

**Nucleotide synthesis in HCV infection.** HCV is an enveloped virus and member of the genus *Hepacivirus* within the Flaviviridae family.<sup>9</sup> It has a single-stranded RNA genome with positive polarity. Within the cell, nucleotides required for RNA and DNA synthesis are made available through one of two pathways: (i) salvage from recycling nucleosides and nucleobases from native RNA and DNA; and (ii) *de novo* synthesis. The rapidity of the cell cycle may necessitate a greater complement of nucleotides than are available by salvage alone and, thus, the *de novo* pathway has been proposed as the primary source of nucleotides for RNA and DNA synthesis in rapidly proliferative cell synthesis (Figure 2).<sup>10,11</sup> In *de novo*

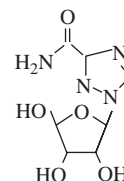
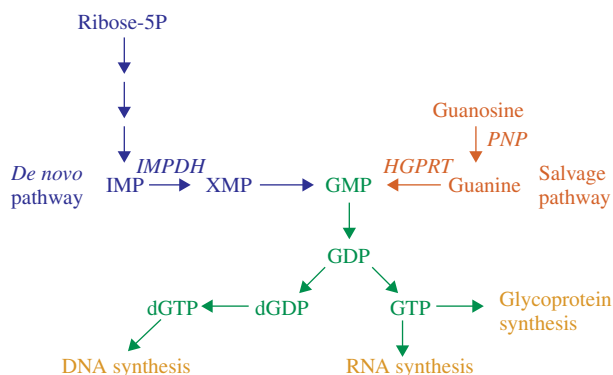


Figure 1. Ribavirin chemical structure.

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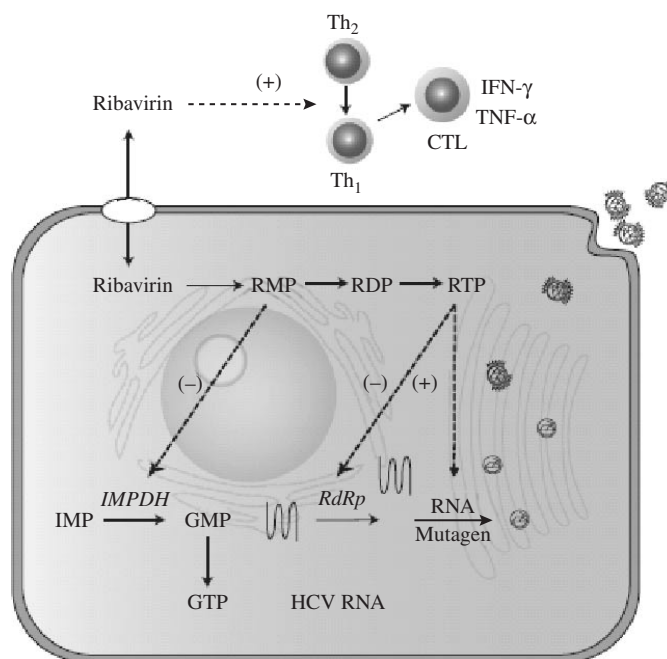


**Figure 2.** IMPDH plays a central role in the synthesis of guanosine nucleosides/nucleotides. IMPDH catalyses the rate-limiting step in guanosine nucleotide biosynthesis. IMPDH inhibition down-regulates the *de novo* production of both RNA and DNA. dGDP, deoxyguanosine diphosphate; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; HGPRT, hypoxanthine guanine phosphoribosyltransferase; IMP, inosine monophosphate; IMPDH, inosine monophosphate dehydrogenase; PNP, purine nucleoside phosphorylase; XMP, xanthosine monophosphate. Reprinted with permission from Gish.<sup>11</sup>

synthesis, IMPDH (EC 1.1.1.205) catalyses the  $\text{NAD}^+$ -dependent conversion of inosine monophosphate into xanthine monophosphate, the rate-limiting step in the biosynthesis of guanine nucleotides. The enzyme has two isoforms: the type I isoform is constitutively expressed, whereas the type II isoform is up-regulated in rapidly proliferating cells.<sup>12</sup> Inhibitors of IMPDH prevent the synthesis of guanosine monophosphate blocking the formation of guanosine nucleotide through the *de novo* pathway.

**Direct mechanisms of action.** Ribavirin enters the cell as a prodrug and is converted into ribavirin 5'-monophosphate (RMP), -diphosphate (RDP) and -triphosphate (RTP) through the sequential actions of three cellular kinases (Figure 3).<sup>8</sup> The actions of RMP, a direct competitive inhibitor of IMPDH, decreases intracellular levels of guanosine triphosphate (GTP). Because GTP is essential for transcription of viral genomes and replication of RNA viruses, low levels of GTP are thought to down-regulate viral replication. Ribavirin inhibits viral guanylyltransferase and mRNA (guanine-7'*N*-)-methyltransferase activity, thereby creating mRNA with abnormal 5'-cap structures and blocking viral transcription.<sup>13</sup> Ribavirin phosphates inhibit viral polymerase; consequently, the enzyme is unable to create copies of the original positive-stranded (antisense) viral RNA.<sup>14</sup> In addition, ribavirin can be incorporated into viral RNA by the polymerase<sup>15</sup> giving rise to ribavirin-induced mutations in the viral genome and replication error (suicide mutations) catastrophe, which results in fewer infectious virions<sup>16</sup> and thus interferes with inter-hepatocyte infection. This raises concerns about the damage to host genetic (chromosomal) structures and probably results in or contributes to the teratogenicity of ribavirin. Ribavirin is also considered as a carcinogen in some animal and *in vivo* models.<sup>7,17</sup>

**Indirect mechanisms of action.** Acute control of HCV RNA levels occurs through a brisk intrahepatic T-helper and T-suppressor cell response, a shift toward a Th<sub>1</sub> cytokine profile and up-regulated natural killer cell activity.<sup>18</sup> This adaptive immune response favours elimination of virus-infected cells. However, as this



**Figure 3.** Immune-mediated and direct antiviral activities of ribavirin. This illustration demonstrates five potential mechanisms of action of ribavirin in patients with chronic hepatitis C: (i) the drug enhances immune clearance of the virus by facilitating the switch from a Th<sub>2</sub> to a Th<sub>1</sub> phenotype with resultant production of antiviral cytokines such as TNF and IFN; (ii) RMP inhibits the enzymatic activity of IMPDH in the conversion of IMP into GMP; (iii) by inhibiting HCV RdRp activity, RTP inhibits HCV RNA replication; (iv) RTP also acts as an RNA mutagen; and (v) the combined direct antiviral activities of Steps (ii)–(iv) result in down-regulation of HCV virion production while HCV particles that are released are defective. CTL, cytotoxic T lymphocytes; HCV, hepatitis C virus; IFN, interferon; IMP, inosine monophosphate; IMPDH, inosine monophosphate dehydrogenase; RDP, ribavirin diphosphate; RdRp, RNA-dependent RNA polymerase; RMP, ribavirin monophosphate; RTP, ribavirin triphosphate; TNF, tumour necrosis factor; RNA, ribonucleic acid.

process continues Th<sub>2</sub> predominance develops, which is associated with infection chronicity.<sup>19</sup> This may be the result of suppressive effects of HCV core proteins on production of interleukin-12, a cytokine essential for induction of Th<sub>1</sub> immunity.<sup>20</sup> Administration of ribavirin shifts the response towards Th<sub>1</sub> cells and their associated cytokines.<sup>21</sup> The resultant Th<sub>1</sub> cytokines, particularly interferon- $\gamma$ , appear to inhibit production of the HCV virion, enhance immunologically mediated lysis of infected hepatocytes, inhibit neoplastic transformation and down-regulate hepatic fibrogenesis.<sup>20</sup> The latter appears to inhibit proliferation and activation of hepatic stellate cells, which are the principal mediators of hepatic collagen formation and extracellular matrix deposition.<sup>22</sup>

**Adverse events**

Ribavirin is associated with haemolytic anaemia that can be either predictable and dose related or unpredictable and potentially dose limiting. Plasma ribavirin is transported into erythrocytes by the 'es' nucleoside transporter and is converted into RMP, RDP and RTP.<sup>23</sup> The ribavirin phosphates accumulate because erythrocytes lack the phosphatases needed to hydrolyse them.<sup>23</sup> Accumulation of the ribavirin phosphates, with a relative deficiency of adenosine triphosphate, produces cellular toxicity and subsequent extravascular haemolysis. Depletion of high energy phosphates is thought

to lead to down-regulation of the hexose monophosphate shunt with an associated increased sensitivity of the erythrocyte to oxidative damage and resultant haemolysis.<sup>24</sup> Ribavirin-related anaemia often occurs rapidly during the first 4 weeks of therapy, when it is crucial to maintain ribavirin levels to maximize chances for an SVR.

In clinical trials, haemoglobin (Hb) levels in pegylated interferon/ribavirin-treated patients decrease by an average of 2–3 g/dL. Approximately 10–13% of patients experience significant anaemia with Hb levels declining below 10 g/dL, the cut point for initiating ribavirin dose reduction in accordance with the ribavirin prescribing information, and up to 52% of patients develop a Hb of <12, the threshold at which oncology patients begin to experience symptoms associated with decreases in quality of life.<sup>25,26</sup> However, because the effect of anaemia on outcomes is a function of factors, such as gender, renal function, geographic elevation, age, comorbidities, activity and rate of Hb decreases, dose reductions may be necessary in patients with Hb levels >10 g/dL. In the pegylated interferon alfa-2a–ribavirin licensing study anaemia prompted ribavirin dose reductions in 22% of patients treated for 48 weeks.<sup>25</sup>

## Ribavirin monotherapy

The efficacy of ribavirin monotherapy in patients with HCV infection has been evaluated in several randomized, double-blind, placebo-controlled trials.<sup>27,28</sup> Results of these and other clinical evaluations<sup>29–31</sup> indicated that ribavirin treatment had modest transient effects on plasma HCV RNA levels but lowered aminotransferase levels. Study results also suggest that ribavirin monotherapy leads to improvement in hepatic histology in some patients who show a biochemical response.<sup>28,29,32</sup> However, the biochemical responses are usually transient and the histological improvements are generally modest. In combination with standard or pegylated interferon, however, ribavirin significantly improves virological outcomes by reducing relapse rates. Therefore, this combination is the current standard of care.<sup>33</sup>

## Ribavirin-like molecules in development

### Viramidine

**Pharmacology.** Viramidine is a liver-targeting, synthetic nucleotide prodrug of ribavirin. Studies of the immunomodulatory activity of the medication indicate that its effect on Th<sub>1</sub> cytokine production and T-cell proliferation is preserved and similar to that of ribavirin.<sup>34</sup> Viramidine is structurally similar to adenosine<sup>35</sup> and can thus be converted into its active form by hepatic adenosine deaminase, a ubiquitous enzyme that catalyses the hydrolysis of adenosine to inosine and ammonia (Figure 4). Both ribavirin and viramidine are rapidly eliminated. Both the parent molecules and metabolites are excreted by the kidneys and have a  $T_{max}$  of

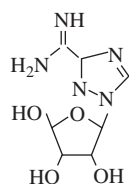


Figure 4. Viramidine chemical structure.

1.5–3 h.<sup>23</sup> Neither medication significantly binds to serum proteins.<sup>36</sup> In non-human primates, the higher viramidine:ribavirin ratio in portal compared with systemic plasma indicates that viramidine is predominantly taken up by the liver (first pass effect) and activated (converted) in the liver to ribavirin by adenosine deaminase.<sup>37</sup> The ribavirin once it is derived from viramidine is subsequently concentrated in the liver. Experimentally, hepatic retention of the ribavirin that is derived from a single oral dose of viramidine is 3-fold greater than that of oral ribavirin. In the same non-human primate model, viramidine produces 50% higher levels of ribavirin in the liver but only one-half in the plasma and red blood cells (RBCs).<sup>23</sup> Because it produces lower RBC levels of ribavirin phosphates, viramidine has the potential to maintain Hb concentrations in patients treated with combination therapy. Studies of cytochrome P450 metabolism in pooled hepatic microsomal fractions indicate that neither medication significantly inhibits or activates the principal human cytochromes.<sup>36</sup> Both viramidine and ribavirin are filtered by the glomeruli and excreted into the urine; however, the amount of either medication measured in urine is only 2–5% indicating that both are predominantly eliminated by metabolism.

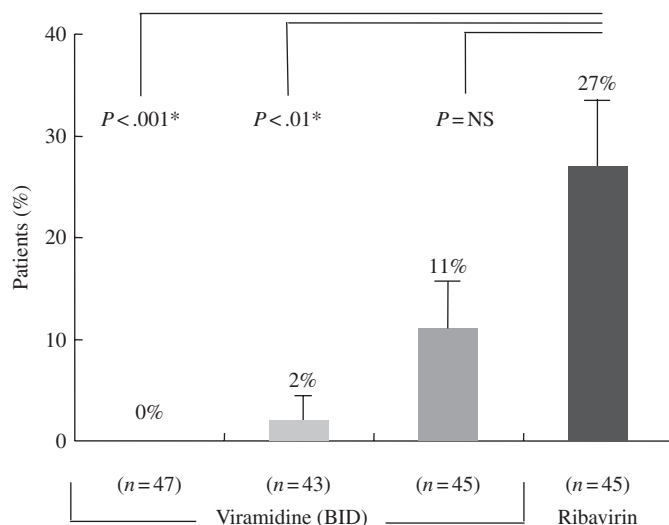
**Adverse events.** Pharmacokinetic and safety studies of viramidine have demonstrated that it is safe and tolerable. Most reported adverse events are mild. The respective percentages of treatment-emergent adverse events that were deemed possibly related to viramidine 200, 600 and 1200 mg were 0, 26 and 50%, respectively.<sup>23</sup> The majority of adverse events were mild and most resolved without sequelae.

**Clinical trial results.** End-of-treatment and SVR results from a Phase 2 randomized, active-controlled, multicentre study of pegylated interferon alfa-2a plus either viramidine or ribavirin in 180 treatment-naïve patients with chronic hepatitis C demonstrated no significant differences between the treatment groups in the proportion of patients with undetectable HCV RNA levels, regardless of HCV genotype during therapy.<sup>38,39</sup> However, significantly fewer patients developed anaemia in the viramidine-treatment groups than in the ribavirin group (4% versus 27%;  $P < 0.001$ ) (Figure 5). No cases of anaemia were reported among patients receiving viramidine 400 mg twice daily, and only one case was reported among those receiving 600 mg twice daily (2%). In contrast, the incidence of defined anaemia was 11% in the viramidine 800 mg twice daily treatment group and 27% in the ribavirin group. Other adverse events were similarly observed between treatment groups.<sup>38,39</sup>

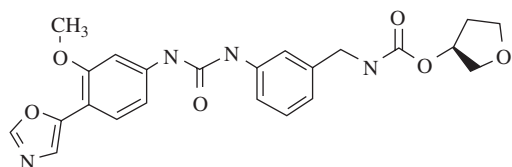
Phase 3 trials of viramidine 600 mg twice daily, known as VISER1 and VISER2 (Viramidine's Safety and Efficacy versus Ribavirin), are currently comparing viramidine plus pegylated interferon alfa-2a or -2b. These studies were designed to determine whether viramidine is as effective as ribavirin and to confirm the medication's erythrocyte-sparing properties—an effect that would remove ribavirin-related anaemia and the associated need for dose modification or recombinant human erythropoietin from the therapeutic equation.

### Merimepodib

Merimepodib (VX-497) is a competitive, oral IMPDH inhibitor with a molecular weight of 452.5 kDa (Figure 6).<sup>10</sup> The medication is a novel, selective inhibitor of IMPDH that was in clinical



**Figure 5.** Safety data from the Phase 2 trial of viramidine in therapy-naïve patients with chronic hepatitis C treated with peginterferon alfa-2a plus viramidine. Patients treated with the 400 and 600 mg twice daily doses of the liver-targeting prodrug of ribavirin had a significantly lower incidence of anaemia (Hb < 10 g/dL) at any time during therapy. \*Adjusted for multiple comparisons.

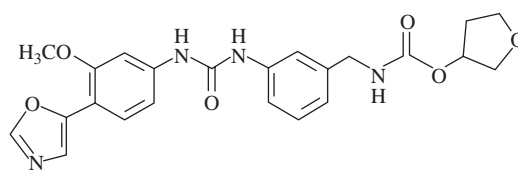


**Figure 6.** Merimepodib chemical structure.

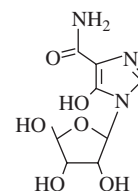
development for the treatment of HCV infection. Merimepodib inhibits both IMPDH isoforms and is structurally unrelated to other medications that show similar catalytic activity. When tested against a variety of DNA and RNA viruses merimepodib demonstrated significant activity.<sup>10</sup> Combination therapy with merimepodib or ribavirin plus interferon alfa showed modest additive activity, with merimepodib more potent in this combination than ribavirin.

**Pharmacology.** Zha *et al.*<sup>40</sup> performed a pharmacokinetic–pharmacodynamic analysis to evaluate the potential relationships between the plasma exposure to merimepodib and ribavirin and the 12-week virological responses or development of anaemia in 31 patients who did not respond to a previous course of pegylated interferon plus ribavirin. Merimepodib or placebo was administered in combination with ribavirin plus pegylated interferon alfa-2a to 31 patients infected with HCV genotype 1. At the end of 12 weeks, 28 patients had completed therapy with pegylated interferon plus weight-based ribavirin in addition to placebo ( $n = 9$ ), merimepodib 25 mg ( $n = 8$ ) or merimepodib 50 mg ( $n = 11$ ) given every 12 h.<sup>40</sup>

Preliminary results showed that the  $AUC_{0-12}$  of merimepodib was significantly higher in patients who showed a virological response than in those who did not.<sup>40</sup> In the logistical regression analysis, age and merimepodib  $AUC_{0-12}$  were the only significant predictors of 12 week virological response. At week 12, a 2-log drop in HCV RNA was reported in 60%, 75% and 80% of patients



**Figure 7.** Mycophenolate mofetil chemical structure.



**Figure 8.** Mizoribine chemical structure.

in the second, third and fourth quartiles of merimepodib plasma drug exposure, respectively, but in no patient in the lowest quartile. The percentage decrease in Hb correlated with trough levels of ribavirin but not the  $AUC_{0-12}$  of merimepodib. These preliminary results supported further studies of triple therapy with these agents.<sup>40</sup>

**Clinical trials.** A Phase 2b trial designed to evaluate two doses of merimepodib added to therapy with pegylated interferon plus ribavirin in non-responders has completed enrolment.<sup>41</sup> The goal of this US trial was to enrol ~315 patients into three study groups at 55 clinical sites. All study patients will receive pegylated interferon alfa-2a plus ribavirin in standard doses. Two groups were to also receive merimepodib twice daily, one group at each dose level being tested and a third group was to receive the pegylated interferon and ribavirin plus placebo. Response to treatment was to be evaluated at 24 weeks, and responders were to receive a total of 48 weeks of therapy. Outcome measures would have included end-of-treatment response, SVR, safety, pharmacokinetics and immunomodulatory activity.

#### Other IMPDH inhibitors

Mycophenolate mofetil is an IMPDH inhibitor used for immunosuppression in the transplantation setting, including orthotopic liver transplantation in patients with chronic hepatitis C (Figure 7). Its immunosuppressive activities appear to far exceed the medication's antiviral properties, although there are currently no ongoing trials to evaluate the medication in this indication.<sup>42</sup> Mycophenolate may be useful for immunosuppression in patients with autoimmune disease as well as autoimmune liver disease and can help with manifestations of chronic hepatitis C.<sup>43</sup> Mizoribine is an IMPDH inhibitor that is marketed as an immunosuppressant (Figure 8). Like mycophenolate, mizoribine has shown antiviral properties but is not currently in clinical trials for treatment of hepatitis C. Mizoribine inhibits the replication of HCV at a concentration (5  $\mu$ M) similar to that reported for ribavirin<sup>44</sup> and thus may find use in anti-HCV regimens in the future.

#### Conclusions

Since the introduction of ribavirin, its ability to inhibit IMPDH has made it a central component in pegylated interferon-based

combination therapy in patients with chronic hepatitis C. However, the haemolytic anaemia associated with ribavirin therapy is potentially dose limiting. Whereas recombinant human erythropoietin can be used to treat the anaemia, its use adds a second medication to treat the complications of the first medication. Experimental studies have demonstrated that other IMPDH inhibitors have antiviral activity. Viramidine, a liver-targeting ribavirin prodrug, has been shown to have end-of-treatment efficacy and SVR comparable to that of ribavirin when combined with pegylated interferon with a substantially lower rate of anaemia. If two large ongoing Phase 3 trials confirm this, viramidine may be used more commonly as the oral antiviral agent in combination regimens with pegylated interferon for the treatment of chronic hepatitis C. While a Phase 2b trial of merimepodib has completed enrolment, preliminary pharmacokinetic–pharmacodynamic results of the anti-HCV activity of merimepodib in combination with pegylated interferon plus ribavirin have resulted in discontinuation of clinical trials. Current data suggest that all other IMPDH inhibitors such as mycophenolate are best used as immunosuppressive therapy.

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