Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Ribavirin: a drug active against many viruses with multiple effects on virus replication and propagation. Molecular basis of ribavirin resistance

Stéphanie Beaucourt and Marco Vignuzzi

Ribavirin has proven to be effective against several viruses in the clinical setting and a multitude of viruses *in vitro*. With up to five different proposed mechanisms of action, recent advances have begun to discern the hierarchy of antiviral effects at play depending on the virus and the host conditions under scrutiny. Studies reveal that for many viruses, antiviral mechanisms may differ depending on cell type *in vitro* and *in vivo*. Further analyses are thus required to accurately identify mechanisms to more optimally determine clinical treatments. In recent years, a growing number of ribavirin resistant and sensitive variants have been identified. These variants not only inform on the specific mechanisms by which ribavirin enfeebles the virus, but also can themselves be tools to identify new antiviral compounds.

Addresses
Institut Pasteur, Viral Populations and Pathogenesis Unit, Centre National de la Recherche Scientifique UMR 3569, 28 rue du Dr Roux, Paris cedex 15, 75724, France

Corresponding author: Vignuzzi, Marco (marco.vignuzzi@pasteur.fr)

Current Opinion in Virology 2014, **8**:10–15

This review comes from a themed issue on **Antivirals and resistance**

Edited by **Luis Menéndez-Arias** and **Douglas D Richman**

For a complete overview see the Issue and the Editorial

Available online 19th May 2014

http://dx.doi.org/10.1016/j.coviro.2014.04.011

Introduction

Ribavirin (RBV) is a synthetic guanosine analog with a broad-spectrum of antiviral activity. It is clinically effective against several viruses, such as respiratory syncytial virus [1], several hemorrhagic fever viruses [2–4] and HCV when combined with pegylated interferon-α [5]. Moreover, it is effective against a large panel of RNA and DNA virus infections *in vitro* (summarized in Table 1). Nevertheless, the mechanism(s) of action of ribavirin are still under scrutiny, appearing to differ depending on the virus in question and the cell line used for study.

Of the proposed mechanisms, two indirectly affect the virus. One of them is based on the inhibition of inosine monophosphate dehydrogenase (IMPDH) by ribavirin-5′-monophosphate, which leads to the depletion of intracellular GTP pools. The second one proposed postulates that unphosphorylated RBV has immunomodulatory effects on antiviral cellular responses, enhancing the T-helper type 1 over type 2 responses, or upregulating the interferon-stimulated response element. In addition, three direct mechanisms have been proposed: first, by inhibition of the viral RNA dependent RNA polymerase (RdRp) through direct interaction with ribavirin-5′-triphosphate; second, by interfering with RNA capping activity (although this has not been extensively studied or demonstrated in recent years); and third, by increasing viral mutation rates through the misincorporation of RBV into the genome, leading to population extinction [6]. Here, we will review recent studies on RBV effects on viral replication and propagation *in vitro* and *in vivo*, and the molecular basis of ribavirin resistance.

Ribavirin and its broad spectrum of antiviral activities

Several recent studies have continued to explore RBV as an antiviral and therapeutic treatment against different viruses. In recent years, these studies have focused on whether RBV is directly inhibiting the RdRp, mutagenizing the genome, inhibiting IMPDH, or a combination of the three.

Inhibition of RNA synthesis is observed for the influenza virus polymerase in cells treated with either RBV or methotrexate, an inhibitor of purine synthesis that decreases intracellular concentrations of purines. In this case, loss of polymerase activity at low concentrations of nucleotide is considered to be the culprit [7]. For hepatitis E virus (HEV) replication *in vitro*, RBV has the same antiviral effect as two other IMPDH inhibitors, mycophenolic acid (MPA) and 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) that can be neutralized by the addition of guanosine. HEV replication appears to be inhibited through the depletion of GTP pools and inhibition of IMPDH [8]. Similarly, RBV's effect on HCV seemingly acts through the inhibition of IMPDH, since the addition of guanosine negates this effect and HCV RNA synthesis is suppressed in IMPDH-knockdown cells. No mutagenic activity was observed for HCV infection of Li23 hepatoma cells [9]. These results are in contradiction with those later obtained in the Huh-7.5 cell line, where mutagenic activity was indeed observed [10,11]. This mutagenesis

Table 1

The broad-spectrum antiviral activity of ribavirin

Family	Genus	Type species
Arenaviridae	Arenavirus	Junin virus, Lassa fever virus, Pichinde virus, Lymphocytic choriomeningitis virus
Bornaviridae	Bornavirus	Borna disease virus V and He/80
Bunyaviridae	Bunyavirus	San Angelo
	Hantavirus	Hantaan virus, Seoul virus
	Nairovirus	Crimean Congo hemorrhagic fever virus
	Phlebovirus	Rift Valley fever virus, Sandy Fever Sicilian virus
Coronaviridae	Coronavirus	Severe Acute Respiratory Syndrome virus, Middle East Respiratory Syndrome virus, murine hepatitis virus
Flaviviridae	Flavivirus	Dengue virus 1, 2, 4, Japanese encephalitis virus, Langat virus, Usutu virus, Wesselsbron virus, West Nile virus, Yellow Fever virus 17D and FNV, Zika virus
	Hepacivirus	GB virus B, Hepatitis C virus
Hepeveridae	Hepevirus	Hepatitis E virus
Orthomyxoviridae	Influenzavirus	Influenza virus A, Influenza virus B
Papillomaviridae	Papillomavirus	Human papillomavirus
Paramyxoviridae	Metapneumovirus	Human Metapneumovirus
	Morbillivirus	Measles virus
	Pneumovirus	Bovine respiratory syncitial virus, Human respiratory syncitial virus
	Respirovirus	Sendai virus
Picornaviridae	Aphtovirus	Foot and mouth disease virus
	Enterovirus	Poliovirus, Coxsackie virus B3, Enterovirus 71, Rhinovirus
Poxviridae	Orthopoxvirus	Camelpox virus, Cowpox virus, Monkeypox virus, Vaccinia virus
Retroviridae	Lentivirus	Human immunodeficiency virus, Maedi Visna virus
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis Indiana virus
Togaviridae	Alphavirus	Chikungunya virus, Semliki Forest virus, Sindbis virus
Unassigned	Deltavirus	Hepatitis delta virus

Adapted and updated from [50]

is not mediated through the depletion of GTP levels, since the addition of guanosine does not significantly alter these effects. Depletion of GTP by MPA also has no mutagenic effect, suggesting that direct incorporation of RBV into the HCV genome is the underlying mechanism. Nevertheless, the study did uncover NTP imbalances resulting from RBV treatment that might contribute to the inhibition of RNA synthesis by mechanisms related to, or independent of, mutagenesis. In the case of arenaviruses, it appears that RBV has multiple effects. With lymphocytic choriomeningitis virus (LMCV), inhibition is likely mediated through a decrease in GTP levels caused by ribavirin-5′-monophosphate inhibition of IMPDH, while a mutagenic activity also occurs through incorporation of RBV into nascent RNA [12•]. Finally, a direct interaction with the viral polymerase was suggested in the case of Hantaan virus (HTNV). In Vero cells treated with RBV, a reduction in viral genomic RNA and infectious particles is observed that is not associated with a significant reduction in GTP levels, as would be expected if IMPDH inhibition were the primary antiviral effect. Rather, an observed increase in ribavirin-5′-triphosphate supports that direct interactions with the RdRp is the mechanism of inhibition [13••,14]. It is likely then, that for most viruses, ribavirin does indeed have pleiotropic effects that may differ in importance depending on concentration and cell type.

These studies highlight the possible contradiction of results due to choice of cell line, and question to what extent cell lines represent *in vivo* settings. Although the study of ribavirin as an antiviral drug has been justly focused its effects on the virus, an important set of recent studies examined how host cells may react to RBV exposure. One study revealed dramatic variations in the accumulation of RBV in seven different cell lines [15••]. The authors showed that while uptake of RBV by the equilibrative nucleoside transporters (ENT1 and 2) is similar for all cell types, the levels of intracellular RBV in seemingly resistant cells like BHK21, A549 and Vero are noticeably lower than those in BSRT7, HeLa, 4T1 or HEp2. Importantly, these profiles correlate with the lack of antiviral efficacy against VSV and Sendai virus. The authors concluded that the ability of a given cell type to metabolize RBV may determine which of RBV's antiviral effects will be at play. In the case of the hCoV-EMC coronavirus, a lack of sensitivity to interferon-α2b and RBV is also observed in Vero cells, while LLC-MK2 cells are more responsive [16•]. Interestingly, RBV-responsive Huh7.5 cells passaged in the presence of ribavirin develop resistance phenotypes that correlate with reduced RBV uptake and/or processing. Furthermore, reduced uptake develops in PBMC of healthy donors treated for 1 week with RBV, and in the PBMC of HCV-infected patients receiving ribavirin-interferon combination therapy [17••].

A trend in recent years has been to explore mutagenesis as the primary antiviral effect against most RNA viruses and lethal mutagenesis is being more seriously considered as a valid antiviral approach [12•,18,19]. However, this trend has been based on biochemical and tissue culture studies; future efforts will likely focus on determining whether mutagenesis occurs *in vivo* and how to optimize this activity in a therapeutic context.

Ribavirin and its clinical use

Although RBV has enough antiviral benefit against some viruses to warrant its clinical use despite its *in vivo* mechanisms remaining unclear, it is possible that this

lack of knowledge results in suboptimal targeting and efficacy.

The standard treatment against non-genotype 1 HCV, until the recent emergence of small molecule direct acting antivirals, has been a combination of pegylated interferon-α and RBV, although neither drug seems to have direct effects on the virus. In this regard, RBV enhances the pSTAT4 and IFN-γ response of natural killer cells to IFN-α stimulation [20]. By assessing RBV physiological levels in mono-infected and HIV co-infected patients, one study correlated lower response rates of HIV-infected patients with lower RBV bioavailability [21]. Interestingly, this combined therapy was surprisingly effective against disseminated human papillomavirus infection in a patient having previously undergone treatment for psoriasis [22], in HIV-HCV co-infected patients [23], as well as in a patient with acquired aplastic anemia [24].

In HCV infected patients undergoing RBV monotherapy, only a moderate and transient antiviral effect is observed. A lack of clear and direct inhibitory/mutagenic effects of RBV on HCV *in vivo* is thought to be a question of physiological concentrations of the drug or an issue of sampling the wrong compartment (blood versus liver). However, in recent studies, RBV was found to induce significantly more G-to-A and C-to-U transitions, a genetic signature that is indicative of ribavirin-induced mutagenesis [25••,26]. Whether the effect is sufficiently strong to be of benefit is not yet determined. For other clinical infections, RBV monotherapy remains the treatment of choice. For example, in transplant patients treated for hepatitis E infection, RBV therapy ensures a sustained virologic response in most patients as a first-line, or as a second and prolonged, therapy [27]. It is also a well-tolerated option to treat respiratory syncytial virus infections in immunocompromised patients [28]. As the only antiviral treatment against Crimean-Congo hemorrhagic fever, it appears to be beneficial when administrated early, with decreased severity and fatality rates [29]. In a preliminary study, combination treatment was also shown to be effective against rhinovirus (RV) in patients with hypogammaglobulinemia, who are more susceptible to RV infections [30]. To explore an intervention strategy for the recently identified β coronavirus isolate hCoV-EMC/2012, treatment of rhesus macaques with RBV alone reduced virus replication. In combination with interferon-α2b, treatment was even more effective and permitted lower doses that are in line with clinical use [31]. Finally, RBV was shown to have protective potential against lethal hantavirus pulmonary syndrome after intranasal exposure of hamsters to Andes Virus, where the presence of live virus was reduced or abolished in serum [32].

Ribavirin and its isolated resistant variants

A number of RBV resistant variants have been either generated or isolated within the *Picornaviridae* family, all mapping to the RdRp and for many of them, resistance was accompanied by increased fidelity (Table 2). The first ribavirin-resistant poliovirus variant contained G64S in the RdRp [33], which led to the generation of four other RBV-resistant variants at this amino acid position (G64A, L,V and T) [34]. These variants resist the effects of several mutagens through increased replication fidelity. Mutations similar to those in poliovirus, G64R and G64T, were generated in human enterovirus 71 (HEV71) that

Table 2

Ribavirin resistant and sensitive variants. Unless otherwise indicated, mutations appear in the RdRp. The mechanism of resistance or sensitivity is indicated, when known

Virus	Variant	RBV phenotype — mechanism	Refs
Poliovirus	G64S/A/L/V/T	Resistant — higher fidelity	[33,34]
FMDV	M296I	Resistant — RBV-specific	[44,45]
	R84H	Resistant — higher fidelity	[36]
	A38V	Resistant — RBV-specific	[43]
	D5N:A38V:M194I:M296V (DAMM)	Resistant — RBV-specific	[43]
Coxsackie B3	A372V	Resistant — higher fidelity	[37]
	P48K, S164P, A239G, L241I	Sensitive — lower fidelity	[46•]
	I176V, I230F, F232Y, Y268W/H	Sensitive — lower fidelity	[46•]
	S299T	Sensitive — lower fidelity	[37]
HEV71	G64R/T	Resistant — higher fidelity	[35]
	L123F	Resistant — higher fidelity	[38]
Chikungunya	C483Y	Resistant — higher fidelity	[39]
	C483M	Resistant — higher fidelity	[47]
	C483A/G/W	Sensitive — lower fidelity	[47]
Sindbis virus	C482A/G	Sensitive — lower fidelity	[47]
HCV	J6/JFH1 resistant populations	Resistant to RBV and 5-FU	[41,42]
	G404S, E442G in NS5A protein	Resistant to RBV	[40]
Coronavirus	MHV-ExoN-	Sensitive — lower fidelity	[51]
	SARS-ExoN-	Sensitive — lower fidelity	[48]
Influenza A	D27N in PB1	Resistant to RBV	[7]

confer resistance to RBV and increased fidelity [35]. Recently, a FMDV mutagen-resistant RdRp variant was isolated using another mutagenic compound, 5-fluorouracil (5-FU). This R84H variant is also resistant to RBV and to a third mutagen, 5-azacytidine (AZC), as would be expected of high fidelity viruses [36]. A naturally occurring RdRp variant A372V of Coxsackie virus B3 is also resistant to these three mutagens through increased fidelity [37]. Yet another high fidelity HEV71 RdRp variant, L123F, was selected under RBV passage [38]. Within the *Togaviridae* family, the polymerase mutant C483Y of chikungunya virus was isolated using mutagenic passaging in RBV and 5-FU [39], which demonstrates higher replication fidelity. For HCV, ribavirin resistant mutations in the NS5A protein were first described using a replicon system [40]. More recently, resistant populations were isolated by serially passaging J6/JFH1 virus in RBV, which present dozens of mutations throughout the genome that could be responsible, including in the RdRp, but the precise determinant is not known [41]. In a later study, they confirmed that this population is also resistant to 5-FU, supporting increased fidelity as the mechanism behind its resistance [42].

In some cases, however, the precise mechanisms of resistance remain unclear and fidelity may or may not be involved. In the *Orthomyxoviridae*, an influenza A virus variant D27N, in the PB1 catalytic subunit of the viral replicase, was isolated that displays higher activity than wild type virus in the presence of RBV, as well as of an inhibitor of purine biosynthesis, methotrexate [7]. The authors suggest that this residue is involved in nucleotide recognition, but whether resistance results from increased fidelity or from increased processivity remains to be determined. Recently, another ribavirin-resistant FMDV polymerase variant A38V and a mutant carrying four RdRp amino acid substitutions called DAMM were isolated, but do not tolerate 5-FU or AZC treatment [43]. These variants have RBV-specific resistance profiles, similar to the first RBV-resistant FMDV polymerase variant M296I [44,45], suggesting that increased fidelity is not the basis of their resistance.

Whereas RBV resistant variants have been widely reported and are more readily isolated by mutagen screening, some RBV sensitive variants have also been recently isolated or generated. A dozen variants in different residue positions of Coxsackie virus B3 polymerase, and several variants of the same RdRp residue of chikungunya and Sindbis virus, are more sensitive to base analog mutagens because of their reduced polymerase fidelity, further confirming the mutagenic affect of RBV on RNA viruses [37,46•,47]. In the case of coronaviruses (CoV) a hyper-sensitive ExoN-mutant has generated interesting data in recent years. Given that coronaviruses have the largest RNA genomes, they would expectedly be hyper-sensitive to RNA mutagens such as RBV; however, this did not seem to be the case. Following the discovery of a potential 3′-to-5′ exoribonuclease (ExoN) proofreading activity of the nsp14 [48], it was hypothesized that deletion of this activity would result in RBV sensitivity. This hypothesis was validated using murine hepatitis virus (MHV) and SARS-CoV ExoN-mutants, treated with RBV or 5-FU [49•].

Concluding remarks

In the last few years, *in vitro* studies on RBV have sought to identify which mechanisms are most contributing to the observed antiviral effect. It appears that in many cases, this pleiotropic compound is simply being true to its pleiotropic nature. A significant recent advance, and complication, toward this goal is the variability observed between cell types and tissues in RBV uptake and metabolism, that could impact the efficacy of a drug being administered with one mechanism in mind, while another mechanism is ultimately at work. Future research requires a better validation of these mechanisms depending on the cell system used, especially in the case of HCV. The most important goal is to determine how ribavirin treatment affects the virus *in vivo*, to more properly tailor treatments and identify alternative compounds with similar effects. Currently, the use of animal models to bridge laboratory and clinical studies are relatively scarce.

Additionally, the recently isolated RBV-resistant and sensitive variants represent new tools to improve the efficacy of current mutagenic compounds and to identify new compounds with previously unknown antiviral mutagenic activity. For example, the isolation of the same Coxsackie virus B3 RdRp variant A372V in two independent screens for RNA mutagen and amiloride resistance, led to the discovery of a previously unknown, indirect mutagenic activity for the latter class of compounds [37]. This is especially relevant in this era of combination therapies, where drugs with different modes of action are needed whether they are inhibitors or mutagens.

Acknowledgements

This work was funded by the Laboratoire d'Excellence 'Integrative Biology of Emerging Infectious Diseases' program (grant n° ANR-10-LABX-62-IBEID) and the Institut Pasteur.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cooper AC, Banasiak NC, Allen PJ: **Management and prevention strategies for respiratory syncytial virus (RSV) bronchiolitis in infants and young children: a review of evidence-based practice interventions**. *Pediatr Nurs* 2003, **29**:452-456.

2. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R: **Lassa fever. Effective therapy with ribavirin**. *N Engl J Med* 1986, **314**:20-26.

3. Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB: **Crimean Congo-haemorrhagic fever treated with oral ribavirin**. *Lancet* 1995, **346**:472-475.

4. Bronze MS, Greenfield RA: **Therapeutic options for diseases due to potential viral agents of bioterrorism**. *Curr Opin Investig Drugs* 2003, **4**:172-178.

5. Pawlotsky J-M, Dahari H, Neumann AU, Hezode C, Germanidis G, Lonjon I, Castera L, Dhumeaux D: **Antiviral action of ribavirin in chronic hepatitis C**. *Gastroenterology* 2004, **126**:703-714.

6. Graci JD, Cameron CE: **Mechanisms of action of ribavirin against distinct viruses**. *Rev Med Virol* 2005, **16**:37-48.

7. Binh NT, Wakai C, Kawaguchi A, Nagata K: **Involvement of the N-terminal portion of influenza virus RNA polymerase subunit PB1 in nucleotide recognition**. *Biochem Biophys Res Commun* 2014, **443**:975-979.

8. Debing Y, Emerson SU, Wang Y, Pan Q, Balzarini J, Dallmeier K, Neyts J: **Ribavirin inhibits in vitro hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with alpha interferon**. *Antimicrob Agents Chemother* 2014, **58**:267-273.

9. Mori K, Ikeda M, Ariumi Y, Dansako H, Wakita T, Kato N: **Mechanism of action of ribavirin in a novel hepatitis C virus replication cell system**. *Virus Res* 2011, **157**:61-70.

10. Ortega-Prieto AM, Sheldon J, Grande-Pérez A, Tejero H, Gregori J, Quer J, Esteban JI, Domingo E, Perales C: **Extinction of hepatitis C virus by ribavirin in hepatoma cells involves lethal mutagenesis**. *PLoS ONE* 2013, **8**:e71039.

11. Perales C, Beach NM, Gallego I, Soria ME, Quer J, Esteban JI, Rice C, Domingo E, Sheldon J: **Response of hepatitis C virus to long-term passage in the presence of alpha interferon: multiple mutations and a common phenotype**. *J Virol* 2013, **87**:7593-7607.

12. • Moreno H, Gallego I, Sevilla N, la Torre de JC, Domingo E, Martín V: **Ribavirin can be mutagenic for arenaviruses**. *J Virol* 2011, **85**:7246-7255.
The inhibitory effect of RBV on IMPDH was shown, as well as its mutagenic effect when incorporated in nascent RNA.

13. •• Sun Y, Chung D-H, Chu Y-K, Jonsson CB, Parker WB: **Activity of ribavirin against Hantaan virus correlates with production of ribavirin-5′-triphosphate, not with inhibition of IMP dehydrogenase**. *Antimicrob Agents Chemother* 2007, **51**:84-88.
The decrease of specific infectivity of HCV was shown to be mediated by RBV, a typical feature of lethal mutagenesis.

14. Chung D-H, Sun Y, Parker WB, Arterburn JB, Bartolucci A, Jonsson CB: **Ribavirin reveals a lethal threshold of allowable mutation frequency for Hantaan virus**. *J Virol* 2007, **81**:11722-11729.

15. •• Shah NR, Sunderland A, Grdzelishvili VZ: **Cell type mediated resistance of vesicular stomatitis virus and Sendai virus to ribavirin**. *PLoS ONE* 2010, **5**:e11265.
Study of resistant and sensitive cell lines, pointing out how the choice of cell type can result in discordant antiviral effects.

16. • Falzarano D, de Wit E, Martellaro C, Callison J, Munster VJ, Feldmann H: **Inhibition of novel β coronavirus replication by a combination of interferon-α2b and ribavirin**. *Sci Rep* 2013, **3**:1686.
The efficacy of combined RBV + interferon treatment was shown on CoV, and the importance of using different cell types to identify mechanism was also highlighted.

17. •• Ibarra KD, Jain MK, Pfeiffer JK: **Host-based ribavirin resistance influences hepatitis C virus replication and treatment response**. *J Virol* 2011, **85**:7273-7283.
Resistance to RBV was shown to develop in the host cell, rather than the virus, in both cell culture and in clinical samples.

18. Perales C, Agudo R, Tejero H, Manrubia SC, Domingo E: **Potential benefits of sequential inhibitor-mutagen treatments of RNA virus infections**. *PLoS Pathog* 2009, **5**:e1000658.

19. Moreno H, Tejero H, la Torre de JC, Domingo E, Martín V: **Mutagenesis-mediated virus extinction: virus-dependent effect of viral load on sensitivity to lethal defection**. *PLoS ONE* 2012, **7**:e32550.

20. Werner JM, Serti E, Chepa-Lotrea X, Stoltzfus J, Ahlenstiel G, Noureddin M, Feld JJ, Liang TJ, Rotman Y, Rehermann B: **Ribavirin improves the IFN-γ response of natural killer cells to IFN-based therapy of hepatitis C virus infection**. *Hepatology* 2014 http://dx.doi.org/10.1002/hep.27092.

21. Soriano V, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, Mauss S, Bräu N, Hatzakis A, Pol S *et al.*: **Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel**. *AIDS* 2007, **21**:1073-1089.

22. Shrestha NK, Hamrock DJ: **Successful treatment of disseminated human papillomavirus infection with pegylated interferon and ribavirin**. *Clin Infect Dis* 2010, **51**:e4-e6.

23. Pavan MHP, Velho PENF, Vigani AG, Gonçalves FL, Aoki FH: **Treatment of human papillomavirus with peg-interferon alfa-2b and ribavirin**. *Braz J Infect Dis* 2007, **11**:383-384.

24. Mosa C, Trizzino A, Trizzino A, Di Marco F, D'Angelo P, Farruggia P: **Treatment of human papillomavirus infection with interferon alpha and ribavirin in a patient with acquired aplastic anemia**. *Int J Infect Dis* 2014 http://dx.doi.org/10.1016/j.ijid.2013.11.021.

25. •• Dietz J, Schelhorn S-E, Fitting D, Mihm U, Susser S, Welker M-W, Füller C, Däumer M, Teuber G, Wedemeyer H *et al.*: **Deep sequencing reveals mutagenic effects of ribavirin during monotherapy of hepatitis C virus genotype 1-infected patients**. *J Virol* 2013, **87**:6172-6181.
Increases in RBV-specific transition mutations in patient samples were shown by deep sequencing, providing evidence of RBV mutagenic activity *in vivo*.

26. Cuevas JM, González-Candelas F, Moya A, Sanjuán R: **Effect of ribavirin on the mutation rate and spectrum of hepatitis C virus in vivo**. *J Virol* 2009, **83**:5760-5764.

27. Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, Radenne S, Coilly A, Garrigue V, D'Alteroche L *et al.*: **Ribavirin for chronic hepatitis E virus infection in transplant recipients**. *N Engl J Med* 2014, **370**:1111-1120.

28. Marcelin JR, Wilson JW, Razonable RR, Mayo Clinic Hematology/Oncology and Transplant Infectious Diseases Services: **Oral ribavirin therapy for respiratory syncytial virus infections in moderately to severely immunocompromised patients**. *Transpl Infect Dis* 2014, **16**:242-250.

29. Ozbey SB, Kader C, Erbay A, Ergönül O: **Early use of ribavirin is beneficial in crimean-congo hemorrhagic fever**. *Vector Borne Zoonotic Dis* 2014, **14**:300-302.

30. Ruuskanen O, Waris M, Kainulainen L: **Treatment of persistent rhinovirus infection with pegylated interferon-α2a and ribavirin in patients with hypogammaglobulinemia**. *Clin Infect Dis* 2014 http://dx.doi.org/10.1093/cid/ciu169.

31. Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, Brining D, Bushmaker T, Martellaro C, Baseler L *et al.*: **Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques**. *Nat Med* 2013, **19**:1313-1317.

32. Ogg M, Jonsson CB, Camp JV, Hooper JW: **Ribavirin protects Syrian hamsters against lethal hantavirus pulmonary syndrome — after intranasal exposure to Andes virus**. *Viruses* 2013, **5**:2704-2720.

33. Pfeiffer JK, Kirkegaard K: **A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity**. *Proc Natl Acad Sci U S A* 2003, **100**:7289-7294.

34. Vignuzzi M, Wendt E, Andino R: **Engineering attenuated virus vaccines by controlling replication fidelity**. *Nat Med* 2008, **14**:154-161.

35. Sadeghipour S, Bek EJ, McMinn PC: **Ribavirin-resistant mutants of human enterovirus 71 express a high replication fidelity phenotype during growth in cell culture**. *J Virol* 2013, **87**:1759-1769.

36. Zeng J, Wang H, Xie X, Yang D, Zhou G, Yu L: **An increased replication fidelity mutant of foot-and-mouth disease virus retains fitness in vitro and virulence in vivo**. *Antiviral Res* 2013, **100**:1-7.

37. Levi LI, Gnädig NF, Beaucourt S, McPherson MJ, Baron B, Arnold JJ, Vignuzzi M: **Fidelity variants of RNA dependent RNA polymerases uncover an indirect, mutagenic activity of amiloride compounds**. *PLoS Pathog* 2010, **6**:e1001163.

38. Meng T, Kwang J: **Attenuation of human enterovirus 71 high-replication-fidelity variants in AG129 mice**. *J Virol* 2014, **88**:5803-5815.

39. Coffey LL, Beeharry Y, Bordería AV, Blanc H, Vignuzzi M: **Arbovirus high fidelity variant loses fitness in mosquitoes and mice**. *Proc Natl Acad Sci U S A* 2011, **108**:16038-16043.

40. Pfeiffer JK, Kirkegaard K: **Ribavirin resistance in hepatitis C virus replicon-containing cell lines conferred by changes in the cell line or mutations in the replicon RNA**. *J Virol* 2005, **79**:2346-2355.

41. Feigelstock DA, Mihalik KB, Feinstone SM: **Selection of hepatitis C virus resistant to ribavirin**. *Virol J* 2011, **8**:402.

42. Mihalik KB, Feigelstock DA: **Sensitivity of a ribavirin resistant mutant of hepatitis C virus to other antiviral drugs**. *PLoS ONE* 2013, **8**:e74027.

43. Zeng J, Wang H, Xie X, Li C, Zhou G, Yang D, Yu L: **Ribavirin-resistant variants of foot-and-mouth disease virus: the effect of restricted quasispecies diversity on viral virulence**. *J Virol* 2014 http://dx.doi.org/10.1128/JVI.03594-13.

44. Arias A, Arnold JJ, Sierra M, Smidansky ED, Domingo E, Cameron CE: **Determinants of RNA-dependent RNA polymerase (in)fidelity revealed by kinetic analysis of the polymerase encoded by a foot-and-mouth disease virus mutant with reduced sensitivity to ribavirin**. *J Virol* 2008, **82**:12346-12355.

45. Ferrer-Orta C, Sierra M, Agudo R, la Higuera de I, Arias A, Perez-Luque R, Escarmis C, Domingo E, Verdaguer N: **Structure of foot-and-mouth disease virus mutant polymerases with reduced sensitivity to ribavirin**. *J Virol* 2010, **84**:6188-6199.

46. • Gnädig NF, Beaucourt S, Campagnola G, Bordería AV, Sanz-Ramos M, Gong P, Blanc H, Peersen OB, Vignuzzi M: **Coxsackievirus B3 mutator strains are attenuated in vivo**. *Proc Natl Acad Sci U S A* 2012 http://dx.doi.org/10.1073/pnas.1204022109.
A panel of low fidelity RdRp variants of a RNA virus were generated, that are hypersensitive to ribavirin and other RNA mutagens.

47. Rozen-Gagnon K, Stapleford KA, Mongelli V, Blanc H, Failloux A-B, Saleh M-C, Vignuzzi M: **Alphavirus mutator variants present host-specific defects and attenuation in mammalian and insect models**. *PLoS Pathog* 2014, **10**:e1003877.

48. Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, Scherbakova S, Graham RL, Baric RS, Stockwell TB *et al.*: **Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing**. *PLoS Pathogens* 2010, **6**:e1000896.

49. • Smith EC, Blanc H, Vignuzzi M, Denison MR: **Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics**. *PLoS Pathog* 2013, **9**:e1003565.
The importance of coronavirus ExoN activity on resisting the effects of RNA mutagens was shown.

50. Vignuzzi M, Stone JK, Andino R: **Ribavirin and lethal mutagenesis of poliovirus: molecular mechanisms, resistance and biological implications**. *Virus Res* 2005, **107**:173-181.

51. Eckerle LD, Lu X, Sperry SM, Choi L, Denison MR: **High fidelity of murine hepatitis virus replication is decreased in nsp14 exoribonuclease mutants**. *J Virol* 2007, **81**:12135-12144.