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## Review article: HCV – STAT-C era of therapy

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### Abbreviations:

Hepatitis C Virus (HCV), Interferon (IFN), rapid virologic response (RVR), early virologic response (EVR), sustained virologic response (SVR), specifically targeted antiviral therapy for hepatitis C (STAT-C), non-structural (NS) protein.

## Summary

Background: Novel, directly acting antiviral agents, also named “specifically targeted antiviral therapy for hepatitis C” (STAT-C) compounds, are currently under development in order to improve treatment opportunities of chronic hepatitis C virus (HCV) infection.

Aim: To review the potential of STAT-C agents which are currently under clinical development with a focus on agents that target HCV proteins.

Methods: Studies evaluating STAT-C compounds were identified by systematic literature search using PubMed as well as databases of abstracts presented in English at recent liver and gastroenterology congresses.

Results: Numerous directly acting antiviral agents are currently under clinical phase I-III evaluation. Final results of phase II clinical trials evaluating the most advanced compounds telaprevir and boceprevir indicate that the addition of these NS3/4A protease inhibitors to pegylated interferon-alfa and ribavirin strongly improves the chance to achieve a SVR in treatment-naive HCV genotype 1 patient as well as in prior non-responders and relapsers to standard therapy. Monotherapy with directly acting antivirals is not suitable since it frequently results in the selection of resistant quasispecies and viral breakthrough. NS5B polymerase inhibitors in general have a lower antiviral efficacy than protease inhibitors and their potency to improve SVR rates remains to be established.

Conclusion: STAT-C compounds in addition to pegylated interferon-alfa and ribavirin are capable to improve SVR rates at least in HCV genotype 1 patients and will therefore be included in future treatment recommendations and guidelines. Future research needs to evaluate whether a SVR can be achieved by combination therapies of STAT-C compounds in interferon-free regimens.

## Introduction

With the current standard of care, a combination therapy of pegylated interferon-alfa plus weight based ribavirin for 24 to 72 weeks, only half of all patients with chronic hepatitis C can be cured<sup>1-4</sup>. The chance to achieve a sustained virologic reponse (SVR) by such regimens differs significantly between HCV genotypes with SVR rates of 40-50% in patients infected with genotype 1, contrasted by SVR rates of approximately 80% in those infected with genotypes 2 or 3<sup>1-5</sup>. In addition, treatment with pegylated interferon- $\alpha$  and ribavirin is long (up to 72 weeks) and associated with numerous side effects like anemia, flu-like symptoms or depression. In view of these facts there is an urgent need for improved treatment strategies. The exploding knowledge of the HCV life cycle and of structural features of the HCV proteins has supported the development of many promising directly acting antiviral agents, also named “specifically targeted antiviral therapy for hepatitis C” (STAT-C) compounds<sup>6-13</sup>. Figure 1 summarizes the HCV life cycle and potential targets for STAT-C<sup>11, 12</sup>. Many of these direct antivirals are currently in phase I-III development and will significantly change treatment options for HCV infection in the near future. The most advanced compounds are telaprevir and boceprevir, which are both inhibitors of the HCV NS3 protease and which have been shown to significantly enhance SVR rates in HCV genotype 1 patients, when applied in addition to pegylated interferon-alfa and ribavirin<sup>14-16</sup>. These and other STAT-C compounds will be described in this review with a focus on agents that were already evaluated in clinical trials (Table 1). Antivirals targeting host proteins which are mandatory for HCV replication (e.g. nitazoxanide, celgosivir or DEBIO-025) are reviewed elsewhere<sup>17-21</sup>.

## Compounds targeting HCV polyprotein procession

### NS3/4A protease inhibitors

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3 The design of NS3/4A inhibitors is relatively difficult because the active site of the NS3/4A  
4 protease is located in a shallow groove between two  $\beta$ -barrels of the protease<sup>6, 7</sup>. Nevertheless,  
5 many NS3/4A protease inhibitors are under development, which in general provide a high  
6 antiviral efficacy but a low genetic barrier to resistance. Protease inhibitors can be divided into  
7 two chemical classes, macrocyclic inhibitors and linear tetra-peptide  $\alpha$ -ketoamid derivatives.  
8 NS3/4A protease inhibitors of both classes strongly inhibit HCV replication during monotherapy,  
9 but also frequently cause the selection of resistant mutants which may be followed by viral  
10 breakthrough<sup>13, 22</sup>. However, it was shown that the frequency of resistance development against  
11 protease inhibitors can be vastly reduced by the additional administration of pegylated interferon  
12 and ribavirin. The most advanced compounds are telaprevir and boceprevir, which are currently  
13 under phase III evaluation and are expected to be approved in 2011/2012.  
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### 29 **Ciluprevir (BILN 2061)**

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32 The first NS3/4A inhibitor applied in clinical studies was ciluprevir (BILN 2061), an orally  
33 bioavailable, peptidomimetic, macrocyclic drug binding non-covalently to the active center of the  
34 enzyme<sup>23</sup>. Ciluprevir monotherapy was evaluated in a double-blind, placebo-controlled pilot  
35 study in treatment-naïve genotype 1 patients with compensated liver disease<sup>24</sup>. In this study,  
36 ciluprevir, administered twice daily for two days at doses ranging from 25 to 500 mg, led to a  
37 mean 2-3 log<sub>10</sub> decrease of HCV RNA serum levels in most patients. Another study with  
38 equivalent design assessed the influence of the HCV genotype on treatment with this protease  
39 inhibitor. Compared to genotype 1 patients, the antiviral activity of ciluprevir was less  
40 pronounced and more variable in patients infected with genotypes 2 or 3<sup>25</sup>. Although the  
41 development of ciluprevir was stopped because of serious cardiotoxicity observed in an animal  
42 model, these studies provided the proof-of-principle for successful suppression of HCV  
43 replication by NS3/4A inhibitors in patients with chronic hepatitis C.  
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### *Viral resistance to ciluprevir*

Because of the high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase, numerous variants (quasispecies) are continuously produced during HCV replication. Among them, variants carrying mutations altering the conformation of the binding sites of STAT-C compounds can develop. During treatment with specific antivirals, these preexisting drug-resistant variants have a fitness advantage and can be selected to become the dominant viral quasispecies. Many of these resistant mutants exhibit an attenuated replication fitness with the consequence that, after termination of exposure to specific antivirals, the wild-type may again replace the resistant variants<sup>22, 26</sup>. Nevertheless, HCV quasispecies resistant to NS3/4A protease inhibitors or non-nucleoside polymerase inhibitors can be detected at very low levels in some patients who were never treated with specific antivirals before<sup>27-29</sup>. The clinical relevance of these pre-existing mutants is not completely understood, although there is evidence that they may reduce the chance to achieve a SVR by therapies based on HCV protease or non-nucleoside polymerase inhibitors.

Exposure of genotype 1 replicon cells to ciluprevir and subsequent sequence analyses of the NS3 region has led to the identification of several mutations conferring ciluprevir-resistance: A156T, R155Q and D168V/A. These mutations result in a 357-fold, 24-fold and 144-fold reduced susceptibility to ciluprevir, respectively, compared to wild-type<sup>30-32</sup>. The A156T mutant confers varying levels of cross-resistance to ciluprevir, telaprevir and boceprevir. The A156T mutation causes a significantly reduced enzymatic function attenuating the HCV life cycle, which, however, can be overcome by additional mutations at P89L, Q86R or G162R<sup>30-32</sup>. No data are available on clinically selected resistance mutations after administration of ciluprevir in patients with chronic hepatitis C.

### **Telaprevir (VX-950)**

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3 Telaprevir is an orally bioavailable NS3 protease inhibitor which belongs to the  $\alpha$ -ketoamids and  
4 binds the enzyme covalently but reversibly, with a half-life of 58 minutes of the enzyme-inhibitor  
5 complex. Currently, telaprevir is under phase III evaluation (ADVANCE- and ILLUMINATE-Study  
6 for treatment-naïve patients, REALIZE study for non-responders).  
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### 11 12 13 14 *Phase I studies*

#### 15 16 *Telaprevir monotherapy study*

17 A double-blind, randomized placebo-controlled phase Ib clinical trial evaluating telaprevir  
18 monotherapy over 14 days was performed in patients with chronic HCV genotype 1 infection<sup>33</sup>.  
19 In this study, antiviral activity, safety, optimal dosage, and pharmacokinetics were assessed in  
20 treatment-naïve patients, relapsers or non-responders to standard treatment. Doses of telaprevir  
21 were 450 mg or 750 mg every 8 hours or 1250 mg every 12 hours. Telaprevir was well tolerated  
22 and led to a rapid decline of HCV RNA serum levels in all groups. The best results were  
23 obtained in the 750 mg telaprevir q8h dose group with a median reduction of HCV RNA of 4.4  
24  $\log_{10}$  after 14 days of treatment, which is the basis for telaprevir-dosage in most of the following  
25 clinical trials. However, viral rebound due to selected mutants occurred in all patients after  
26 treatment completion and in some patients even during therapy. The selection of resistant  
27 mutants was more frequent in patients who received suboptimal doses<sup>22</sup>.  
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#### 45 *Telaprevir / pegylated (peg) interferon $\alpha$ -2a / ribavirin combination studies*

46 A second phase I study investigated the safety, viral kinetics and the development of telaprevir-  
47 resistant mutants of telaprevir monotherapy and in combination with pegIFN  $\alpha$ -2a in treatment-  
48 naïve genotype 1 patients<sup>34</sup>. Telaprevir dosage was 750 mg every 8 hours after an initial loading  
49 dose of 1250 mg and it was administered either alone or in combination with pegIFN  $\alpha$ -2a in  
50 comparison to pegIFN  $\alpha$ -2a monotherapy. Treatment was given for 14 days and caused a  
51 median reduction of HCV RNA of 1.09  $\log_{10}$  in the pegIFN  $\alpha$ -2a/placebo group, of 3.99  $\log_{10}$  in  
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3 the telaprevir/placebo group and of 5.49 log<sub>10</sub> in the telaprevir/pegIFN  $\alpha$ -2a group at the end of  
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5 therapy. As observed before, selection of telaprevir-resistant mutants occurred during telaprevir  
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7 monotherapy. However, their frequency was significantly lower during combination therapy with  
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9 pegIFN  $\alpha$ -2a and no viral breakthrough was seen during the combination therapy within 14  
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11 days<sup>34</sup>.

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14 A parallel study evaluated the safety and efficacy of telaprevir (750 mg every 8 hours) in  
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16 combination with pegIFN  $\alpha$ -2a and weight-based ribavirin in treatment-naïve genotype 1 patients  
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18 for 28 days<sup>35</sup>. At the end of the 28-day treatment period, all patients had undetectable HCV RNA  
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20 serum levels.  
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### 23 24 25 *Phase II studies*

#### 26 27 *Telaprevir and peg interferon with and without ribavirin*

#### 28 29 *Studies in treatment naïve patients (PROVE 1 and 2, C208, C209, C210)*

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32 Larger phase II clinical trials (PROVE 1 and 2) in treatment naïve genotype 1 patients assessed  
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34 whether with additional telaprevir to pegIFN  $\alpha$ -2a and ribavirin overall treatment duration can be  
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36 reduced and / or SVR rates be improved (Figure 2 and 3). PROVE 1 was conducted in the USA  
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38 whereas PROVE 2 was conducted in Europe. In addition, a study comparing two versus three  
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40 times daily administration of telaprevir in combination with either pegylated interferon alfa 2a or  
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42 2b (C208) and studies in genotype 2, 3 and 4 infected patients were performed (C209, C210).  
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46 In PROVE 1, telaprevir, pegIFN  $\alpha$ -2a and ribavirin were administered for 12 weeks in  
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48 combination, followed by pegIFN  $\alpha$ -2a and ribavirin alone for 0 (n=17), 12 (n=79) or 36 (n=79)  
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50 weeks in comparison to standard treatment. SVR rates were 35%, 61% and 67%, respectively,  
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52 compared to 41% with standard treatment. According to the study protocol, treatment was only  
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54 stopped after 12 or 24 weeks when a rapid virological response (RVR) was achieved. Serious  
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56 adverse effects led to premature treatment termination in 18% of all subjects treated with  
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3 telaprevir in contrast to 4% of patients with standard-therapy. Most common adverse events  
4  
5 were skin rash, anemia and gastrointestinal disorders<sup>15</sup>.  
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8 The study design of PROVE 2 was similar to PROVE 1 with the main difference being that  
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10 treatment termination after 12 or 24 weeks was independent of achieving an RVR and one  
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12 treatment arm was ribavirin-free. The recently published final results showed SVR rates of 36%,  
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14 60% and 69% for patients treated with telaprevir plus pegIFN alone for 12 weeks (n=78),  
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16 telaprevir and pegIFN and ribavirin for 12 weeks (n=82), and with telaprevir, pegIFN and ribavirin  
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18 for 12 weeks followed by 12 weeks of pegIFN plus ribavirin alone (n=81), respectively. The SVR  
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20 rate achieved by standard treatment was 46%. However, the rate of relapse in the groups  
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22 treated for 12 weeks was relatively high with 30% and 48% of all patients who were treated with  
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24 and without ribavirin, respectively. Two patients who discontinued treatment at day 60 and 65  
25  
26 experienced a late relapse 36 and 48 weeks after the end of treatment, respectively<sup>14</sup>.  
27

28  
29 The results of PROVE 1 and 2 indicate that 12 weeks of triple therapy was too short because of  
30  
31 the high rate of relapse after treatment completion. Moreover, ribavirin is necessary in therapies  
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33 with telaprevir to achieve high SVR rates. However, 24 to 48 weeks of total therapy including 12  
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35 weeks of triple therapy with telaprevir in addition to standard treatment greatly improved SVR  
36  
37 rates in treatment-naïve genotype 1 patients compared to the standard of care. The RVR during  
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39 triple therapy is an important predictor for treatment success and can be applied for defining  
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41 individualized treatment durations.  
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44 The most important side effects of telaprevir are rash, gastrointestinal disorders and anemia.  
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46 Although severe rash may require treatment discontinuation, moderate forms can be treated  
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48 successfully with topical steroids. The median decline of blood hemoglobin concentration related  
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50 to telaprevir was approximately 1g/dl. Since telaprevir was administered in most trials for only 12  
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52 weeks, the use of erythropoietin-analogs was rarely necessary.  
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3 C208 was a small study (n=161) comparing three times daily 750mg with two times daily  
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5 1125mg telaprevir combined with pegylated interferon alfa 2a or 2b, respectively, and ribavirin<sup>36</sup>.  
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7 In all 4 treatment arms comparable SVR rates were observed (81-85%). These high overall SVR  
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9 rates underline the potential of the triple therapy approach. They are explained in part by  
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11 experienced study centers with very low discontinuation rates (5%) in comparison with the  
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13 PROVE studies. In addition, in this study the response-guided therapy approach was  
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15 investigated. Treatment duration was shortened to 24 weeks in patients who achieved a RVR,  
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17 while the remaining patients received 48 weeks therapy. Between 80% - 83% of all patients  
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19 treated with pegIFN alfa 2a, and 67% - 69% of all patients treated with pegIFN alfa 2b achieved  
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21 an RVR and could therefore be treated for 24 weeks.  
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27 Because the amino acid sequence of the NS3 protease domain varies significantly between  
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29 HCV genotypes, protease inhibitors may have a different antiviral efficacy in patients infected  
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31 with different genotypes. Like ciluprevir, telaprevir alone or in combination with pegIFN and  
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33 ribavirin was less effective in treatment-naïve patients infected with other genotypes than  
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35 genotype 1. For HCV genotype 2, a somewhat weaker antiviral activity in comparison with HCV  
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37 genotype 1 was observed with a mean viral decline of 3.9 log<sub>10</sub> IU/ml during 14 days of  
38  
39 monotherapy with telaprevir. In genotype 3 and 4 infected patients no significant antiviral activity  
40  
41 was detectable (0.5-0.9 log<sub>10</sub> decline)<sup>37, 38</sup>.  
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#### 46 *Studies in non-responders and relapsers (PROVE 3)*

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48 The PROVE 3 trial was conducted to determine SVR rates of treatment with telaprevir in  
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50 combination with pegIFN-alfa and ribavirin in treatment-experienced patients (Figure 4).  
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52 Telaprevir was administered in combination with pegIFN  $\alpha$ -2a with and without ribavirin for 12 to  
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54 24 weeks followed by pegIFN  $\alpha$ -2a and ribavirin alone for up to 24 weeks. Retreatment of  
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56 previous non-responders with 12 weeks of triple therapy followed by 12 weeks of standard  
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3 treatment led to a SVR rate of 51% (69% Relapser, 39% Non-Responder), which is significantly  
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5 higher compared to SVR rates achieved with the standard of care (14%). Retreatment of non-  
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7 responders with 24 weeks of triple therapy followed by 24 weeks of standard treatment led to a  
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9 SVR rate of 53% (76% Relapser, 38% Non-Responder) and retreatment of non-responders with  
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11 24 weeks of telaprevir and pegIFN- $\alpha$ 2a without ribavirin followed by 24 weeks of pegIFN- $\alpha$ 2a  
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13 alone led to a SVR rate of only 24% (42% Relapser, 11% Non-Responder). The latter result  
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15 indicates that ribavirin is required for a successful treatment of non-responders with telaprevir.  
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17 As in the PROVE 1 and 2 studies viral breakthrough was observed more frequently in patients  
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19 infected with genotype 1a than in patients infected with genotype 1b<sup>39</sup>.  
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### 24 25 *Phase III studies*

#### 26 27 *Design of phase III clinical trials: telaprevir with pegylated interferon-alfa and ribavirin*

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29 Phase III clinical trials evaluating telaprevir in combination with pegIFN-alfa and ribavirin have  
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31 been initiated. The ADVANCE trial enrolled more than 1000 treatment-naïve HCV genotype 1  
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33 patients to evaluate 24 weeks of telaprevir-based therapy. Telaprevir was dosed at 750 mg  
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35 every 8 hours and given for 8 or 12 weeks in combination with pegIFN- $\alpha$ -2a and ribavirin  
36  
37 followed by pegIFN- $\alpha$ -2a and ribavirin alone until treatment week 24. Patients who did not  
38  
39 achieve an RVR were treated with pegIFN- $\alpha$ -2a and ribavirin until week 48. In the ILLUMINATE  
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41 trial telaprevir was given for 12 weeks in combination with pegIFN- $\alpha$ -2a and ribavirin followed by  
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43 pegIFN- $\alpha$ -2a and ribavirin alone until treatment week 24 or 28. The aim of the ILLUMINATE trial  
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45 is to assess whether treatment extension beyond 24 weeks of total therapy improves SVR rates  
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47 in patients with RVR or EVR. The REALIZE study enrolled more than 650 patients with prior  
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49 failure to standard treatment. PegIFN- $\alpha$ -2a and ribavirin was given for 48 weeks including 12  
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51 weeks of telaprevir at a dose of 750 mg every eight hours. In one treatment arm, telaprevir  
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53 treatment was initiated after a 4 week lead-in phase of pegIFN- $\alpha$ -2a and ribavirin alone. SVR  
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55 data of the ADVANCE, ILLUMINATE and REALIZE study are expected to be published in 2010.  
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### *Viral resistance to telaprevir*

To date, mutations conferring telaprevir-resistance have been identified at four positions, V36A/M/L, T54A, R155K/M/S/T and A156S//T<sup>22, 30, 31, 40</sup>, see Table 2 and 3. The A156 mutation was shown by in vitro analyses in the replicon assay while the other mutations could only be detected in vivo by a clonal sequencing approach during telaprevir administration in patients with chronic hepatitis C. A detailed kinetic analysis of telaprevir-resistant variants was performed in genotype 1 patients during 14 days of telaprevir monotherapy and combination therapy with pegIFN  $\alpha$ -2a<sup>41</sup>. Telaprevir monotherapy initially led to a rapid HCV RNA decline in all patients due to a strong reduction in wild-type virus. In patients who developed a viral rebound during telaprevir monotherapy, mainly the single mutation variants R155K/T and A156/T were uncovered by wild-type reduction and became dominant after day 8. These single mutation variants were selected from preexisting quasispecies. During the viral rebound phase these variants typically were replaced by highly resistant double-mutation variants (e.g., V36M/A +R155K/T). The combination of telaprevir and pegIFN  $\alpha$ -2a was sufficient to inhibit the breakthrough of resistant mutations in a 14-day study. It is important to note that after up to 3 years after telaprevir treatment low to medium levels of V36 and R155 variants were still observed in single patients<sup>42</sup>. Another study modeling the dynamics of wild type HCV genotype 1 in patients treated with telaprevir with and without pegylated interferon-alfa and ribavirin showed a first and second phase reduction in virus decline which was up to 10-fold stronger than reported for the standard of care<sup>43</sup>.

As shown for other NS3/4A protease inhibitors as well (e.g. ITMN-191), the genetic barrier to telaprevir resistance differs significantly between HCV subtypes. In all clinical studies of telaprevir alone or in combination with pegIFN-alfa and ribavirin, viral resistance and breakthrough occurred much more frequently in patients infected with HCV genotype 1a compared to genotype 1b. This difference was shown to result from nucleotide differences at

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2  
3 position 155 in HCV subtype 1a (aga, encodes R) versus 1b (cga, also encodes R). The  
4  
5 mutation most frequently associated with resistance to telaprevir is R155K; changing R to K at  
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7 position 155 requires 1 nucleotide change in HCV subtype 1a and 2 nucleotide changes in  
8  
9 subtype 1b isolates<sup>44</sup>.  
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### 11 12 13 14 **Boceprevir (SCH 503034)**

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16 Boceprevir is another novel peptidomimetic orally bioavailable  $\alpha$ -ketoamid HCV protease  
17  
18 inhibitor that forms a covalent but reversible complex with the NS3 protein<sup>45</sup>. Like telaprevir,  
19  
20 boceprevir is currently in phase III evaluation.  
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#### 23 24 25 *Phase I studies*

##### 26 27 *Boceprevir monotherapy study*

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29 An initial phase I trial evaluated safety, tolerability and antiviral efficacy of boceprevir  
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31 monotherapy (100 to 400 mg daily) in HCV genotype 1 patients with prior failure to standard  
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33 therapy<sup>46</sup>. After the 14-day treatment period, a mean  $\log_{10}$  reduction in HCV RNA load of 2.06  
34  
35 was achieved in patients treated with 400 mg boceprevir daily. Boceprevir was well tolerated at  
36  
37 all doses without significant adverse effects. However, viral breakthrough with selection of  
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39 resistant variants occurred in some patients with a frequency depending on boceprevir dosage<sup>47</sup>.  
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##### 45 46 *Boceprevir / peg interferon $\alpha$ -2b combination study*

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48 A subsequent phase Ib study evaluated the combination of boceprevir and pegIFN  $\alpha$ -2b in HCV  
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50 genotype 1-infected non-responders to standard therapy<sup>48</sup>. In this randomized, double-blind  
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52 crossover study, boceprevir was administered at doses of 200 or 400 mg every eight hours  
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54 either alone for seven days or in combination with pegIFN  $\alpha$ -2b for 14 days in comparison to 14  
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56 days of pegIFN  $\alpha$ -2b monotherapy. Because HCV genotype 1 non-responders to standard  
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3 treatment are heterogeneous, the study design intended each patient to receive boceprevir  
4 alone, in combination with pegIFN  $\alpha$ -2b and pegIFN  $\alpha$ -2b alone with washout-periods in  
5 between in a randomized crossover sequence. Mean maximum reductions in HCV RNA load  
6 were 2.45 and 2.88  $\log_{10}$  for boceprevir 200 mg and 400 mg plus pegIFN  $\alpha$ 2b, 1.08 and 1.61  
7  $\log_{10}$  for boceprevir monotherapy and 1.08 and 1.26  $\log_{10}$  for pegIFN  $\alpha$ 2b monotherapy.  
8 Boceprevir was well-tolerated alone and in combination with pegIFN  $\alpha$ 2b. Viral breakthrough  
9 due to selection of preexisting resistant mutants was observed in some patients, in particular  
10 during boceprevir monotherapy<sup>49</sup>.  
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### 23 *Phase II studies*

#### 24 *Boceprevir and peg interferon with and without ribavirin*

##### 25 *Treatment naïve phase II study (SPRINT-1)*

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28 The aim of the SPRINT 1 trial was to investigate safety, tolerability and antiviral efficacy of  
29 boceprevir (800 mg three times a day) in combination with pegIFN  $\alpha$ 2b and ribavirin in  
30 treatment-naïve HCV genotype 1 patients<sup>16</sup>. Treatment with boceprevir in combination with  
31 pegIFN  $\alpha$ 2b and ribavirin was either performed continuously for 28 or 48 weeks or for 24 or 44  
32 weeks after a previous 4-week lead-in phase of pegIFN  $\alpha$ 2b and ribavirin alone. The lead-in  
33 design was chosen to determine a potential benefit of pretreatment with pegIFN- $\alpha$ 2b and  
34 ribavirin on avoiding resistance development. The control group was treated with pegIFN- $\alpha$ 2b  
35 and ribavirin for 48 weeks. SVR rates after 28 weeks of triple treatment were 54% and 56% after  
36 24 weeks with an additional 4 weeks of pretreatment lead in with pegIFN  $\alpha$ 2 and ribavirin (Figure  
37 5). SVR rates after 48 weeks of triple treatment were 67% and 75% after 44 weeks with an  
38 additional 4 weeks of pretreatment lead in with pegIFN- $\alpha$ 2b and ribavirin. After 4 weeks triple  
39 therapy with boceprevir, pegIFN and ribavirin 38% of patients achieved an RVR. The most  
40 common side-effects related to boceprevir were anemia, nausea, vomiting and dysgeusia. In  
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3 general, SPRINT-1 has proven a higher antiviral efficacy of combination therapy with boceprevir  
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5 in comparison to the standard of care with slightly better results after a 4 week lead-in phase.  
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7 However, RVR rates of only 38% during boceprevir triple therapy indicate that boceprevir is  
8  
9 potentially less potent than telaprevir which, during triple therapy with pegIFN- $\alpha$ 2b, lead to an  
10  
11 RVR rate of approximately 70%.

#### 12 13 14 15 16 *Studies in non-responders and relapsers*

17  
18 In a complex study of HCV genotype 1 non-responders, the addition of boceprevir to pegIFN  
19  
20  $\alpha$ 2b and ribavirin resulted in only slightly increased SVR rates compared to standard treatment  
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22 (14% versus 2%)<sup>50</sup>.

#### 23 24 25 26 27 *Design of phase III studies*

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29 A phase III clinical trial (SPRINT-2) evaluating boceprevir in treatment-naïve patients was  
30  
31 initiated recently and has enrolled more than 1000 patients. Equivalent to the SPRINT-1 study  
32  
33 design, patients receive 800 mg boceprevir three times daily in combination with pegIFN- $\alpha$ 2b  
34  
35 and weight based ribavirin for 28 or 48 weeks. RESPOND-2 evaluates boceprevir in combination  
36  
37 with pegIFN- $\alpha$ 2b and ribavirin at the same doses but for 36 and 48 weeks in relapsers and non-  
38  
39 responders. In all investigational arms a lead-in strategy with pegIFN- $\alpha$ 2b and ribavirin is  
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41 followed.  
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#### 45 46 47 *Viral resistance to boceprevir*

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49 In the replicon system, mutations at three positions conferring boceprevir resistance were  
50  
51 discovered (Table 3). T54A, A156S and V170A confer low level resistance to boceprevir  
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53 whereas A156T, which also confers telaprevir and ciluprevir resistance, exhibited greater levels  
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55 of resistance<sup>26</sup>. In patients with chronic hepatitis C three additional mutations were detected  
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57 during boceprevir monotherapy (V36G/M/A, V55A, R155K)<sup>47</sup>. In a number of these patients one  
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3 year and in single patients even 4 years after the end of boceprevir treatment still resistant  
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5 variants were detected in the HCV quasispecies by clonal sequence analysis<sup>51</sup>. However, an  
6  
7 additional study revealed that the antiviral activity of boceprevir was not impaired in patients who  
8  
9 were treated with boceprevir with and without pegIFN-alfa before<sup>49</sup>.

### 14 **Other NS3 protease inhibitors**

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17 Other NS3 protease inhibitors are currently in phase 1-2 development (R7227/ITMN191,  
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19 MK7009, BI201335, TMC435350, SCH900518, BMS-650032, PHX1766, ACH-1625)<sup>13, 52, 53</sup>. In  
20  
21 general, they exhibit a high antiviral activity in HCV genotype 1 patients, comparable to telaprevir  
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23 and boceprevir (Figure 6). Triple therapy studies for a number of compounds have been initiated  
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25 and confirm that resistance development is significantly reduced by combination with pegylated  
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27 interferon and ribavirin. Whereas linear tetrapeptide and macrocyclic inhibitors do not differ in  
28  
29 general with respect to their antiviral activity, their resistance profile differs significantly.  
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31 However, R155 is an overlapping position for resistance and different mutations at this amino  
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33 acid site within the NS3 protease confer resistance to all protease inhibitors which are currently  
34  
35 in advanced clinical development<sup>13</sup>.

### 40 **NS4A inhibitors**

#### 43 **ACH-806**

46  
47 NS4A is a crucial cofactor of NS3, mandatory for proper folding of the protease and capable to  
48  
49 enhance the enzymatic activity of NS3 manifold. ACH-806 targets NS4A and therefore inhibits  
50  
51 the NS3/4A protease by a different mechanism than peptidomimetic NS3 inhibitors. ACH-806  
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53 binds to newly synthesized NS4A molecules, which leads to the blockade of their assembly with  
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55 NS3 proteins. A phase Ib trial in HCV genotype 1-infected patients demonstrated that ACH-806  
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57 has a significant inhibitory impact on HCV replication<sup>54</sup>. Although the development of ACH-806

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3 was halted due to reversible serum creatinine elevations, the concept of NS4A inhibition was  
4 proven. Importantly, no cross-resistance between ACH-806 and peptidomimetic NS3/4A  
5 protease inhibitors was observed in vitro<sup>55, 56</sup>. Novel NS4A inhibitors (e.g. ACH-1095) are  
6 currently under preclinical development.  
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## 11 12 13 **Compounds targeting HCV replication**

### 14 15 **NS5B polymerase inhibitors**

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17 NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analog  
18 inhibitors (NIs) like valopicitabine (NM283), R7128, R1626, PSI-7851 or IDX184 mimic the  
19 natural substrates of the polymerase and are incorporated into the growing RNA chain, thus  
20 causing direct chain termination by tackling the active site of NS5B<sup>29, 57-67</sup>. Because the active  
21 centre of NS5B is a highly conserved region of the HCV genome, NIs are potentially effective  
22 against different genotypes, in contrast to NS3/4A inhibitors. Moreover, single amino acid  
23 substitutions in every position of the active centre may result in loss of function. Thus, there is a  
24 relatively high genetic barrier in the development of resistances to NIs.  
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36 In contrast to NIs, the heterogeneous class of non-nucleoside inhibitors (NNIs) bind to different  
37 allosteric enzyme sites, which results in conformational protein change before the elongation  
38 complex is formed<sup>68</sup>. To inhibit NS5B allosterically, a high chemical affinity of the compound to  
39 the enzyme is required. NS5B is structurally organized in a characteristic “right hand motif”,  
40 containing finger, palm and thumb domains, and offers at least four NNI-binding sites, a  
41 benzimidazole-(thumb 1)-, thiophene-(thumb 2)-, benzothiadiazine-(palm 1)- and benzofuran-  
42 (palm 2)-binding site<sup>68, 69</sup>. Theoretically, NNIs targeting different binding sites can be used in  
43 combination or in sequence to manage the development of resistance. Because NNIs bind  
44 distantly to the active centre of NS5B, their application results more frequently in resistance  
45 development than during treatment with NIs. In addition, mutations at the NNI-binding sites do  
46 not necessarily lead to impaired function of the enzyme.  
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## Nucleoside analogs

Valopicitabine (NM283, 2'-C-methylcytidine/NM107) was the first nucleoside inhibitor investigated in patients with chronic hepatitis C. Antiviral activity of valopicitabine was low<sup>70</sup>. The clinical development of valopicitabine was stopped due to gastrointestinal side effects and an insufficient risk / benefit profile.

The second nucleoside inhibitor investigated in patients with chronic hepatitis C was R1626 (4'-azidocytidine/PSI-6130). A phase 1 study showed a high antiviral activity at high doses of R1626 in patients infected with HCV genotype 1<sup>63-65</sup>. No viral breakthrough with selection of resistant variants was reported from monotherapy or combination studies with pegylated interferon ± ribavirin. However, severe lymphopenia and infectious disease adverse events led to the stop of R1626 development.

R7128 is another nucleoside polymerase inhibitor with potent antiviral activity during monotherapy in HCV genotype 1 patients. Currently, R7128 is investigated in phase 2 clinical trials in HCV genotype 1, 2 and 3 infected patients in combination with pegylated interferon and ribavirin<sup>59</sup>. Both during monotherapy and combination therapy with pegylated interferon and ribavirin no resistance development against R7128 was observed.

Other nucleoside analog inhibitors of the NS5B polymerase (PSI-7851 and IDX184) are evaluated in phase 1 clinical trials in patients with chronic hepatitis C and many compounds are under preclinical development<sup>13</sup>. For a summary of antiviral activities of nucleoside polymerase inhibitors see Figure 7.

## Non-nucleoside analogs

### *NNI-site 1 inhibitors (thumb 1 / benzimidazole site)*

BILB1941, BI207127 and MK-3281 are NNI-site 1 inhibitors which have been investigated in clinical phase 1 trials and exhibit low to medium antiviral activities<sup>13, 71, 72</sup>. No selection of

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3 resistant variants and viral breakthrough has been observed during 5 days of treatment with  
4 BILB1941 or BI207127.  
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10 *NNI-site 2 inhibitors (thumb 2 / thiophene site)*

11 Filibuvir (PF-00868554) is a NNI-site 2 inhibitor with medium antiviral activity in a phase 1 study.  
12 In a subsequent trial viral breakthrough was observed in 5 of 26 patients during combination  
13 therapy with pegIFN  $\alpha$ -2a and ribavirin for 4 weeks<sup>72</sup>.  
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16 Other NNI-site 2 inhibitors which were evaluated in phase 1 trials are VCH-759, VCH-916 and  
17 VCH-222, their antiviral efficacy is shown in Figure 8<sup>13, 73</sup>. Like during treatment with filibuvir,  
18 VCH-759 and VCH-916 application resulted in viral breakthroughs with selection of resistant  
19 variants, indicating a low genetic barrier to resistance of these agents.  
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29 *NNI-site 3 inhibitors (palm 1 / benzothiadiazine site)*

30 ANA598 is a NNI-site 3 inhibitor which displayed antiviral activity during treatment of HCV  
31 genotype 1 infected patients. No viral breakthrough was observed during a short term  
32 monotherapy trial<sup>74</sup>.  
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40 *NNI-site 4 inhibitors (palm 2 / benzofuran site)*

41 Monotherapy with the NNI-site 4 inhibitor HCV-796 showed low antiviral activity in HCV  
42 genotype 1 infected patients and resulted in selection of resistant variants and viral breakthrough  
43 in several patients<sup>75, 76</sup>. GS-9190 displays a low antiviral activity in a clinical study and variants  
44 conferring resistance were identified in the beta-hairpin of the polymerase.  
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53 An overview of the antiviral activities of non-nucleoside polymerase inhibitors in monotherapy  
54 studies is shown in Figure 8.  
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### NS5A inhibitors

In a single ascending dose study it was shown that inhibition of the NS5A protein with BMS-790052 leads to a sharp initial decline of HCV RNA concentrations<sup>77</sup>. BMS-790052 binds to domain I of the NS5A protein, which was shown to be important for regulation of HCV replication. No clinical data on resistance to this class of drugs have been presented yet and results of multiple dose and combination therapy studies have to be awaited.

### NS4B inhibitors

NS4B is a hydrophobic protein mandatory for the formation of the membranous web of the HCV replication complex. Moreover, NS4B displays RNA-binding properties which may be crucial in HCV RNA procession and replication. In vitro, inhibition of NS4B by small molecular compounds has been shown to compromise HCV replication significantly<sup>78</sup>.

### Combination therapies of specific antivirals

It is a fundamental question whether SVR can be achieved by combination therapies of different STAT-C compounds without pegIFN-alfa and ribavirin. A first clinical trial (INFORM-1 study) evaluated the combination of a polymerase inhibitor (R7128) and a NS3 inhibitor (R7227/ITMN191). In this proof of principle study, patients were treated with both compounds for up to 2 weeks. HCV RNA concentrations decreased up to 5.2 log<sub>10</sub> IU/ml, no viral breakthrough was observed, and HCV RNA was undetectable at the end of dosing in up to 63% of treatment-naïve patients<sup>79</sup>. Future clinical trials need to address whether a long-term suppression of HCV replication or even SVR can be achieved with such direct antiviral combination therapies. Currently, combination studies with several compounds are conducted (R7128+R7227, VX-950+VCH222, BMS790052+BMS650032, BI201335+BI207127).

## Conclusions

Numerous directly acting antiviral agents are currently under clinical phase I-III evaluation. Results of phase II clinical trials evaluating the most advanced compounds telaprevir and boceprevir indicate that the addition of these NS3/4A protease inhibitors to pegylated interferon- $\alpha$  and ribavirin substantially improves the chance to achieve a SVR in treatment-naïve HCV genotype 1 patients as well as in prior non-responders and relapsers to standard therapy. In addition, at least during treatment with telaprevir-based regimens, overall treatment durations can be shortened significantly.

Results of the milestone studies PROVE 1 and 2 indicate that 12 weeks of telaprevir-based triple therapy is too short because of the high rate of relapse after treatment completion. Moreover, ribavirin is necessary in therapies with telaprevir to achieve high SVR rates. However, 24 to 48 weeks of total therapy including 12 weeks of triple therapy with telaprevir in addition to standard treatment greatly improved SVR rates in treatment-naïve genotype 1 patients compared to the standard of care. The RVR during triple therapy is an important predictor for treatment success and can be applied for defining individualized treatment durations. Important side effects of telaprevir are, as observed during treatment with other protease inhibitors as well, anemia, rash and gastrointestinal disorders. The SPRINT-1 trial demonstrated that SVR rates in treatment-naïve HCV genotype 1 patients can be enhanced by the addition of boceprevir to standard treatment as well. However, the lower antiviral efficacy of boceprevir compared to telaprevir may require longer durations of boceprevir application.

PROVE 3 has shown that telaprevir is also highly effective in the treatment of prior non-responders or relapsers infected with HCV genotype 1. In contrast, addition of boceprevir to standard treatment only revealed a minor impact on SVR rates in non-responders, but further trials are awaited. In addition to telaprevir and boceprevir, many NS3/4A inhibitors with promising antiviral activities are currently investigated in phase I and II trials.

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3 Compared to NS3/4A protease inhibitors, most HCV polymerase inhibitors display a lower  
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5 antiviral activity during monotherapy. SVR data of triple therapies containing NS5B inhibitors  
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7 need to be awaited. However, some polymerase inhibitors are equally effective against different  
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9 HCV genotypes whereas it was shown that protease inhibitors such as telaprevir are less potent  
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11 in other genotypes than HCV genotype 1. In addition, NS5B inhibitors at least of the nucleoside  
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13 analog family display a high genetic barrier to resistance.  
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17 Though it can be vastly reduced by addition of pegylated interferon-alfa and ribavirin, resistance  
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19 development to directly acting antiviral agents has to be kept in mind. R155 is the overlapping  
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21 mutation conferring resistance to all clinically yet evaluated protease inhibitors. Although  
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23 resistance against polymerase inhibitors needs to be better characterized, it is evident that their  
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25 resistance profiles differ from those of protease inhibitors. Combination of different classes of  
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27 STAT-C agents may therefore help to overcome limitations of resistance development. The  
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29 impact of recently discovered polymorphisms near the IL28B gene on resistance development  
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31 and SVR rates during STAT-C regimens needs to be characterized in future studies<sup>80-82</sup>.  
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35 A pivotal trial evidenced an additive antiviral efficacy of the polymerase inhibitor R7128 in  
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37 combination with the protease inhibitor ITMN-191 in an interferon- and ribavirin-free regimen.  
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39 Whether SVR can be achieved with such interferon-free regimens needs to be addressed in  
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41 future trials.

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43 In conclusion, STAT-C compounds in addition to pegylated interferon-alfa and ribavirin are  
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45 capable to improve SVR rates at least in HCV genotype 1 patients and will therefore be included  
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47 in future treatment recommendations and guidelines.  
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**References**

1. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.
2. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-65.
3. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;339:1485-92.
4. Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, Sarrazin C, Harvey J, Brass C, Albrecht J. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004;40:993-9.
5. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-55.
6. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MD, Landro JA, Chambers SP, Markland W, Lepre CA, O'Malley ET, Harbeson SL, Rice CM, Murcko MA, Caron PR, Thomson JA. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 1996;87:343-55.
7. Kim JL, Morgenstern KA, Griffith JP, Dwyer MD, Thomson JA, Murcko MA, Lin C, Caron PR. Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. *Structure* 1998;6:89-100.
8. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, Rice CM. Complete replication of hepatitis C virus in cell culture. *Science* 2005;309:623-6.
9. Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999;285:110-3.
10. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005;11:791-6.
11. Bartenschlager R, Frese M, Pietschmann T. Novel insights into hepatitis C virus replication and persistence. *Adv Virus Res* 2004;63:71-180.
12. Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007;5:453-63.
13. Sarrazin C, Zeuzem S. Resistance to Direct Antiviral Agents in Patients With Hepatitis C Virus Infection. *Gastroenterology* 2009;138:447-462.



14. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourliere M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009;360:1839-50.
15. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-38.
16. Kwo P, Lawitz E, McCone J, Schiff E, Vierling J, Pound D, Davis M, Galati J, Gordon S, Ravendhran N, Rossaro L, Anderson F, Jacobson I, Rubin R, Koury K, Brass C, Chaudhri E, Albrecht J. HCV SPRINT-1 final results: SVR 24 from a phase 2 study of boceprevir plus peginterferon alfa-2b/ribavirin in treatment-naive subjects with genotype-1 chronic hepatitis C. *J Hepatol* 2009;50(Suppl. 1):4.
17. Flisiak R, Horban A, Gallay P, Bobardt M, Selvarajah S, Wiercinska-Drapalo A, Siwak E, Cielniak I, Higersberger J, Kierkus J, Aeschlimann C, Grosgrin P, Nicolas-Metral V, Dumont JM, Porchet H, Crabbe R, Scalfaro P. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfectd with hepatitis C and human immunodeficiency virus. *Hepatology* 2008;47:817-26.
18. Khattab MA. Targeting host factors: a novel rationale for the management of hepatitis C virus. *World J Gastroenterol* 2009;15:3472-9.
19. Rossignol JF, Kabil SM, El-Gohary Y, Keeffe EB. Randomized controlled trial of nitazoxanide-peginterferon-ribavirin, nitazoxanide-peginterferon and peginterferon-ribavirin in the treatment of patients with chronic hepatitis c genotype 4. *J Hepatol* 2008;48:30.
20. Rossignol JF, Elfert A, Keeffe EB. Evaluation of a 4 week lead-in phase with nitazoxanide prior to nitazoxanide+peginterferon in treating chronic hepatitis C. *J Hepatol* 2008;48:311.
21. Schinazi RF, Bassit L, Gavegnano C. HCV drug discovery aimed at viral eradication. *J Viral Hepat* 2009.
22. Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, Wincheringer D, Zhou Y, Chu HM, Lin C, Weegink C, Reesink H, Zeuzem S, Kwong AD. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroenterology* 2007;132:1767-77.
23. Lamarre D, Anderson PC, Bailey M, Beaulieu P, Bolger G, Bonneau P, Bos M, Cameron DR, Cartier M, Cordingley MG, Faucher AM, Goudreau N, Kawai SH, Kukolj G, Lagace L, LaPlante SR, Narjes H, Poupert MA, Rancourt J, Sentjens RE, St George R, Simoneau B, Steinmann G, Thibeault D, Tsantrizos YS, Weldon SM, Yong CL, Llinas-Brunet M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* 2003;426:186-9.
24. Hinrichsen H, Benhamou Y, Wedemeyer H, Reiser M, Sentjens RE, Calleja JL, Fornis X, Erhardt A, Cronlein J, Chaves RL, Yong CL, Nehmiz G, Steinmann GG. Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. *Gastroenterology* 2004;127:1347-55.
25. Reiser M, Hinrichsen H, Benhamou Y, Reesink HW, Wedemeyer H, Avendano C, Riba N, Yong CL, Nehmiz G, Steinmann GG. Antiviral efficacy of NS3-serine protease inhibitor BILN-2061 in patients with chronic genotype 2 and 3 hepatitis C. *Hepatology* 2005;41:832-5.

- 1
- 2
- 3 26. Tong X, Chase R, Skelton A, Chen T, Wright-Minogue J, Malcolm BA. Identification and analysis
- 4 of fitness of resistance mutations against the HCV protease inhibitor SCH 503034. *Antiviral Res*
- 5 2006;70:28-38.
- 6
- 7 27. Gaudieri S, Rauch A, Pfafferott K, Barnes E, Cheng W, McCaughan G, Shackel N, Jeffrey GP,
- 8 Mollison L, Baker R, Furrer H, Gunthard HF, Freitas E, Humphreys I, Klenerman P, Mallal S,
- 9 James I, Roberts S, Nolan D, Lucas M. Hepatitis C virus drug resistance and immune-driven
- 10 adaptations: relevance to new antiviral therapy. *Hepatology* 2009;49:1069-82.
- 11
- 12 28. Kuntzen T, Timm J, Berical A, Lennon N, Berlin AM, Young SK, Lee B, Heckerman D, Carlson J,
- 13 Reyor LL, Kleyman M, McMahon CM, Birch C, Schulze Zur Wiesch J, Ledlie T, Koehrsen M,
- 14 Kodira C, Roberts AD, Lauer GM, Rosen HR, Bihl F, Cerny A, Spengler U, Liu Z, Kim AY, Xing Y,
- 15 Schneidewind A, Madey MA, Fleckenstein JF, Park VM, Galagan JE, Nusbaum C, Walker BD,
- 16 Lake-Bakaar GV, Daar ES, Jacobson IM, Gomperts ED, Edlin BR, Donfield SM, Chung RT, Talal
- 17 AH, Marion T, Birren BW, Henn MR, Allen TM. Naturally occurring dominant resistance mutations
- 18 to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. *Hepatology*
- 19 2008;48:1769-78.
- 20
- 21 29. Le Pogam S, Sessaadri A, Kang H, Kosaka A, Hu S, Symons J, Klumpp K, Cammack N, Najera I.
- 22 Low level of resistance, low viral fitness and absence of resistance mutations at baseline
- 23 quasispecies may contribute to high barrier to R1626 resistance in vivo. *J Hepatol* 2008;48:10A.
- 24
- 25 30. Lin C, Gates CA, Rao BG, Brennan DL, Fulghum JR, Luong YP, Frantz JD, Lin K, Ma S, Wei YY,
- 26 Perni RB, Kwong AD. In vitro studies of cross-resistance mutations against two hepatitis C virus
- 27 serine protease inhibitors, VX-950 and BILN 2061. *J Biol Chem* 2005;280:36784-91.
- 28
- 29 31. Lin K, Kwong AD, Lin C. Combination of a hepatitis C virus NS3-NS4A protease inhibitor and
- 30 alpha interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in
- 31 replicon cells. *Antimicrob Agents Chemother* 2004;48:4784-92.
- 32
- 33 32. Lu L, Pilot-Matias TJ, Stewart KD, Randolph JT, Pithawalla R, He W, Huang PP, Klein LL, Mo H,
- 34 Molla A. Mutations conferring resistance to a potent hepatitis C virus serine protease inhibitor in
- 35 vitro. *Antimicrob Agents Chemother* 2004;48:2260-6.
- 36
- 37 33. Reesink HW, Zeuzem S, Weegink CJ, Forestier N, van Vliet A, van de Wetering de Rooij J,
- 38 McNair L, Purdy S, Kauffman R, Alam J, Jansen PL. Rapid decline of viral RNA in hepatitis C
- 39 patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. *Gastroenterology*
- 40 2006;131:997-1002.
- 41
- 42 34. Forestier N, Reesink HW, Weegink CJ, McNair L, Kieffer TL, Chu HM, Purdy S, Jansen PL,
- 43 Zeuzem S. Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with
- 44 hepatitis C. *Hepatology* 2007;46:640-8.
- 45
- 46 35. Lawitz E, Rodriguez-Torres M, Muir AJ, Kieffer TL, McNair L, Khunvichai A, McHutchison JG.
- 47 Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis
- 48 C patients. *J Hepatol* 2008;49:163-9.
- 49
- 50 36. Marcellin P, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, Drenth JP, DeBacker K,
- 51 vanHeeswijk R, Luo D, Gaston P, Beumont-Mauviel M. Virological analysis of patients receiving
- 52 telaprevir administered q8h or q12h with peginterferon-alfa-2a or -alfa2b and ribavirin in
- 53 treatment-naive patients with genotype 1 hepatitis C: study C208. *Hepatology* 2009;50(Suppl
- 54 1):395.
- 55
- 56 37. Benhamou Y, Moussalli J, Ratzu V, Lebray P, Gysen V, DeBacker K, Ghys A, vanHeeswijk R,
- 57 Vangeneugden T, Picchio G, Beumont-Mauviel M. Results of a prove of concept study (C210) of
- 58
- 59
- 60

- 1  
2  
3 telaprevir monotherapy and in combination with peginterferon alfa-2a and ribavirin in treatment-  
4 naive genotype 4 HCV patients. *J Hepatol* 2009;50(Suppl. 1):6.  
5  
6 38. Foster GR, Hezode C, Bronowicki JP, Carosi G, Weiland O, Verlinden L, vanHeeswijk R,  
7 Vangeneugden T, Picchio G, Beumont-Mauviel M. Activity of telaprevir alone or in combination  
8 with peginterferon alfa-2a and ribavirin in treatment-naive genotype 2 and 3 hepatitis-C patients:  
9 interim results of study C209. *J Hepatol* 2009;50(Suppl. 1):22.  
10  
11 39. McHutchison JG, Manns M, Muir A, Terrault N, et al. Retreatment of HCV patients with telaprevir,  
12 peginterferon and ribavirin. *NEJM* 2010, in press.  
13  
14 40. Lin K, Perni RB, Kwong AD, Lin C. VX-950, a novel hepatitis C virus (HCV) NS3-4A protease  
15 inhibitor, exhibits potent antiviral activities in HCV replicon cells. *Antimicrob Agents Chemother*  
16 2006;50:1813-22.  
17  
18 41. Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD, Zeuzem S.  
19 Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C  
20 virus replication in patients. *Hepatology* 2007;46:631-9.  
21  
22 42. Forestier N, Susser S, Welker MW, Karey U, Zeuzem S, Sarrazin C. Long term follow-up of  
23 patients previously treated with telaprevir. *Hepatology* 2008;48(Suppl. 1):760.  
24  
25 43. Adiwijaya BS, Hare B, Caron PR, Randle JC, Neumann AU, Reesink HW, Zeuzem S, Herrmann  
26 E. Rapid decrease of wild-type hepatitis C virus on telaprevir treatment. *Antivir Ther* 2009;14:591-  
27 5.  
28  
29 44. McCown MF, Rajyaguru S, Kular S, Cammack N, Najera I. GT-1a or GT-1b subtype-specific  
30 resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. *Antimicrob Agents*  
31 *Chemother* 2009;53:2129-32.  
32  
33 45. Malcolm BA, Liu R, Lahser F, Agrawal S, Belanger B, Butkiewicz N, Chase R, Gheyas F, Hart A,  
34 Hesk D, Ingravallo P, Jiang C, Kong R, Lu J, Pichardo J, Prongay A, Skelton A, Tong X,  
35 Venkatraman S, Xia E, Girijavallabhan V, Njoroge FG. SCH 503034, a mechanism-based inhibitor  
36 of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral  
37 activity of alpha interferon in replicon cells. *Antimicrob Agents Chemother* 2006;50:1013-20.  
38  
39 46. Zeuzem S, Sarrazin C, Wagner F, et al. Antiviral activity of SCH 503034, a HCV protease  
40 inhibitor, administered as monotherapy in hepatitis C genotype 1 (HCV-1) patients refractory to  
41 pegylated interferon (PEG-IFN-alpha). *Hepatology* 2005;42:276A.  
42  
43 47. Susser S, Welsch C, Wang Y, Zettler M, Domingues FS, Karey U, Hughes E, Ralston R, Tong X,  
44 Herrmann E, Zeuzem S, Sarrazin C. Characterization of resistance to the protease inhibitor  
45 boceprevir in hepatitis C virus-infected patients. *Hepatology* 2009;50:1709-18.  
46  
47 48. Sarrazin C, Rouzier R, Wagner F, Forestier N, Larrey D, Gupta SK, Hussain M, Shah A, Cutler D,  
48 Zhang J, Zeuzem S. SCH 503034, a novel hepatitis C virus protease inhibitor, plus pegylated  
49 interferon alpha-2b for genotype 1 nonresponders. *Gastroenterology* 2007;132:1270-8.  
50  
51 49. Vermehren J, Susser S, Karey U, Forestier N, et al. Clonal analysis of mutations selected in the  
52 NS3 protease domain of genotype 1 non-responders sequentially treated with boceprevir  
53 (SCH503034) and/or pegylated interferon-alfa-2b (pegIFN- $\alpha$ -2b). *Hepatology* 2009;50(Suppl. 4):  
54 1040.  
55  
56 50. Schiff E, Poordard F, Jacobson I, Flamm S, Bacon B, Lawitz E, Gordon S, McHutchison J, Ghalib  
57 R, Poynard T, Sulkowski M, Trepo C, Rizzetto M, Zeuzem S, Marcellin P, Mendez P, Brass C,

- 1  
2  
3 Albrecht JK. Boceprevir combination therapy in null responders: response dependent on  
4 interferon responsiveness. *J Hepatol* 2008;48(Suppl. 2):46.  
5  
6 51. Susser S, Forestier N, Welker MW, Vermehren J, Karey U, Zeuzem S, Sarrazin C. Detection of  
7 Resistant Variants in the Hepatitis C Virus NS3 Protease Gene by Clonal Sequencing at Long-  
8 Term Follow-Up in Patients Treated with Boceprevir. *J Hepatol* 2009;(Suppl1).50:7.  
9  
10 52. Reesink HW, Fanning GC, Farha KA, Weegink C, Van Vliet A, Van 't Klooster G, Lenz O, Aharchi  
11 F, Marien K, Van Remoortere P, Kock HD, Broeckaert F, Meyvisch P, Van Beirendonck E,  
12 Simmen K, Verloes R. Rapid HCV-RNA Decline With Once Daily TMC435: A Phase I Study in  
13 Healthy Volunteers and Hepatitis C Patients. *Gastroenterology* 2009.  
14  
15 53. Forestier N, Larrey D, Guyader D, Marcellin P, Rouzier R, Patel AA, Bradford WZ, Porter S,  
16 Zeuzem S. Treatment of chronic hepatitis C virus (HCV) genotype 1 patients with the NS3/4A  
17 protease inhibitor ITMN-191 leads to rapid reductions in plasma HCV RNA: results of a phase 1b  
18 multiple ascending dose study. *Hepatology* 2008;48(Suppl):1132.  
19  
20 54. Pottage JC, Lawitz E, Mazur D, Wyles D, Vargas H, Ghalib R, al. e. Short-term antiviral activity  
21 and safety of ACH-806, an NS4A antagonist, in HCV genotype 1 infected individuals. *J Hepatol*  
22 2007;46.  
23  
24 55. Wyles DL, Kaihara KA, Schooley RT. Synergy of a Hepatitis C Virus (HCV) NS4A Antagonist in  
25 Combination with HCV Protease and Polymerase Inhibitors. *Antimicrob Agents Chemother*  
26 2008;52:1862-4.  
27  
28 56. Yang W, Zhao Y, Fabrycki J, Hou X, Nie X, Sanchez A, al. e. Selection of Replicon Variants  
29 Resistant to ACH-806, a Novel Hepatitis C Virus Inhibitor with No Cross-Resistance to NS3  
30 Protease and NS5B Polymerase Inhibitors. *Antimicrob Agents Chemother* 2008;52:2043-52.  
31  
32 57. Ali S, Leveque V, Le Pogam S, Ma H, Philipp F, Inocencio N, Smith M, Alker A, Kang H, Najera I,  
33 Klumpp K, Symons J, Cammack N, Jiang WR. Selected replicon variants with low-level in vitro  
34 resistance to the hepatitis C virus NS5B polymerase inhibitor PSI-6130 lack cross-resistance with  
35 R1479. *Antimicrob Agents Chemother* 2008;52:4356-69.  
36  
37 58. Klumpp K, Leveque V, Le Pogam S, Ma H, Jiang WR, Kang H, Granycome C, Singer M, Laxton  
38 C, Hang JQ, Sarma K, Smith DB, Heindl D, Hobbs CJ, Merrett JH, Symons J, Cammack N, Martin  
39 JA, Devos R, Najera I. The novel nucleoside analog R1479 (4'-azidocytidine) is a potent inhibitor  
40 of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture. *J Biol Chem*  
41 2006;281:3793-9.  
42  
43 59. Lalezari J, Gane J, Rodriguez-Torres M, DeJesus E, Nelson D, Everson G, Jacobson I, al. e.  
44 Potent antiviral activity of the HCV nucleoside polymerase inhibitor R7128 with peg-IFN and  
45 ribavirin: interim results of R7128 500mg bid for 28 days. *J Hepatol* 2008;48:29.  
46  
47 60. Le Pogam S, Jiang WR, Leveque V, Rajyaguru S, Ma H, Kang H, Jiang S, Singer M, Ali S,  
48 Klumpp K, Smith D, Symons J, Cammack N, Najera I. In vitro selected Con1 subgenomic  
49 replicons resistant to 2'-C-methyl-cytidine or to R1479 show lack of cross resistance. *Virology*  
50 2006;351:349-59.  
51  
52 61. Nelson D, Pockros PJ, Godofsky E, Rodriguez-Torres M, Everson G, Fried M, al. e. High end-of-  
53 treatment response (84%) after 4 weeks of R1626, peginterferon alfa-2a (40kd) and ribavirin  
54 followed by a further 44 weeks of peginterferon alfa-2a and ribavirin. *J Hepatol* 2008;48:371.  
55  
56 62. Pierra C, Benzaria S, Amador A, Moussa A, Mathieu S, Storer R, Gosselin G. Nm 283, an efficient  
57 prodrug of the potent anti-HCV agent 2'-C-methylcytidine. *Nucleosides Nucleotides Nucleic Acids*  
58 2005;24:767-70.  
59  
60

- 1
- 2
- 3 63. Pockros P, Nelson D, Godofsky E, Rodriguez-Torres M, Everson GT, Fried MW, Ghalib R,  
4 Harrison S, Nyberg L, Shiffman ML, Chan A, Hill G. High relapse rate seen at week 72 for patients  
5 treated with R1626 combination therapy. *Hepatology* 2008;48:1349-50.  
6
- 7 64. Pockros PJ, Nelson D, Godofsky E, Rodriguez-Torres M, Everson GT, Fried MW, Ghalib R,  
8 Harrison S, Nyberg L, Shiffman ML, Najera I, Chan A, Hill G. R1626 plus peginterferon Alfa-2a  
9 provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in  
10 combination with ribavirin. *Hepatology* 2008;48:385-97.  
11
- 12 65. Roberts SK, Cooksley G, Dore GJ, Robson R, Shaw D, Berns H, Hill G, Klumpp K, Najera I,  
13 Washington C. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized,  
14 placebo-controlled study in patients with chronic hepatitis C. *Hepatology* 2008;48:398-406.  
15
- 16 66. Koch U, Narjes F. Allosteric inhibition of the hepatitis C virus NS5B RNA dependent RNA  
17 polymerase. *Infect Disord Drug Targets* 2006;6:31-41.  
18
- 19 67. Koch U, Narjes F. Recent progress in the development of inhibitors of the hepatitis C virus RNA-  
20 dependent RNA polymerase. *Curr Top Med Chem* 2007;7:1302-29.  
21
- 22 68. Beaulieu PL. Non-nucleoside inhibitors of the HCV NS5B polymerase: progress in the discovery  
23 and development of novel agents for the treatment of HCV infections. *Curr Opin Investig Drugs*  
24 2007;8:614-34.  
25
- 26 69. Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC. Crystal structure of the  
27 RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. *Nat*  
28 *Struct Biol* 1999;6:937-43.  
29
- 30 70. Lawitz E, Nguyen T, Younes Z. Clearance of HCV RNA with valopicitabine plus peginterferon  
31 treatment-naive patients with HCV-1 infection: Results at 24 and 48 weeks. *J Hepatol* 2007;46:9.  
32
- 33 71. Erhardt A, Deterding K, Benhamou Y, Reiser M, Forns X, Pol S, Calleja JL, Ross S, Spangenberg  
34 HC, Garcia-Samaniego J, Fuchs M, Enriquez J, Wiegand J, Stern J, Wu K, Kukolj G, Marquis M,  
35 Beaulieu P, Nehmiz G, Steffgen J. Safety, pharmacokinetics and antiviral effect of BILB 1941, a  
36 novel hepatitis C virus RNA polymerase inhibitor, after 5 days oral treatment. *Antivir Ther*  
37 2009;14:23-32.  
38
- 39 72. Shi ST, Herlihy KJ, Graham JP, Nonomiya J, Rahavendran SV, Skor H, Irvine R, Binford S,  
40 Tatlock J, Li H, Gonzalez J, Linton A, Patick AK, Lewis C. Preclinical characterization of PF-  
41 00868554, a potent nonnucleoside inhibitor of the hepatitis C virus RNA-dependent RNA  
42 polymerase. *Antimicrob Agents Chemother* 2009;53:2544-52.  
43
- 44 73. Cooper C, Lawitz E, Ghali P, al. et. Evaluation of VCH-759 monotherapy in hepatitis C infection. *J*  
45 *Hepatol*;51:39-46.  
46
- 47 74. Thompson PA, Patel R, Showalter RE, Li C, Appleman JR, Steffy K. In vitro studies demonstrate  
48 that combinations of antiviral agents that include HCV polymerase inhibitor ANA598 have the  
49 potential to overcome viral resistance. *Hepatology* 2008;48(Suppl):1164.  
50
- 51 75. Kneteman NM, Howe AY, Gao T, Lewis J, Pevear D, Lund G, Douglas D, Mercer DF, Tyrrell DL,  
52 Immermann F, Chaudhary I, Speth J, Villano SA, O'Connell J, Collett M. HCV796: A selective  
53 nonstructural protein 5B polymerase inhibitor with potent anti-hepatitis C virus activity in vitro, in  
54 mice with chimeric human livers, and in humans infected with hepatitis C virus. *Hepatology*  
55 2009;49:745-52.  
56  
57  
58  
59  
60

- 1  
2  
3 76. Villano SA, Raible D, Harper D, Speth J, Chandra P, Shaw P, Bichier G. Antiviral activity of the  
4 non-nucleoside polymerase inhibitor, HCV-796, in combination with pegylated interferon alfa-2b in  
5 treatment-naive patients with chronic HCV. *J Hepatol* 2007;46.  
6  
7 77. Nettles R, Chien C, Chung E, Persson A, Gao M, Belema M, Meanwell NA, DeMicco M, Marbury  
8 TC, Goldwater R, Northup P, Coumbis J, Kraft WK, Charlton MR, Lopez-Talavera JC, Grasela D.  
9 BMS-790052 is a first-in-class potent hepatitis C virus NS5A inhibitor for patients with chronic  
10 HCV infection: results from a proof-of-concept study. *Hepatology* 2008;48(Suppl):1025.  
11  
12 78. Einav S, Gerber D, Bryson PD, Sklan EH, Elazar M, Maerkl SJ, Glenn JS, Quake SR. Discovery  
13 of a hepatitis C target and its pharmacological inhibitors by microfluidic affinity analysis. *Nat*  
14 *Biotechnol* 2008;26:1019-27.  
15  
16 79. Gane EJ, Roberts SK, Stedman C, Angus PW, Ritchie B, Elston R, Ipe D, Baher L, Morcos P,  
17 Najera I, Mannino M, Brennan B, Berrey M, Bradford W, Yetzer E, Shulman N, Smith PF. First-in-  
18 man demonstration of potent antiviral activity with a nucleoside polymerase (R7128) and protease  
19 (R7227/ITMN-191) inhibitor combination in HCV: safety, pharmacokinetics, and virologic results  
20 from INFORM-1. *J Hepatol* 2009;50(Suppl. 1):380.  
21  
22 80. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen  
23 AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts  
24 hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.  
25  
26 81. Rauch A, Kutalik Z, Descombes P, Cai T, di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi  
27 E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Gunthard HF, Heim M, Hirschel B,  
28 Malinverni R, Moradpour D, Mullhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F,  
29 Telenti A, Bochud PY. Genetic variation in IL28B Is Associated with Chronic Hepatitis C and  
30 Treatment Failure - A Genome-Wide Association Study. *Gastroenterology* 2010, epub.  
31  
32 82. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander  
33 G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG,  
34 Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C  
35 virus. *Nature* 2009;461:798-801.  
36  
37 83. Barth H, Liang TJ, Baumert TF. Hepatitis C virus entry: molecular biology and clinical implications.  
38 *Hepatology* 2006;44:527-35.  
39  
40 84. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R,  
41 Nicosia A, Cosset FL. Cell entry of hepatitis C virus requires a set of co-receptors that include the  
42 CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 2003;278:41624-30.  
43  
44 85. Lozach PY, Amara A, Bartosch B, Virelizier JL, Arenzana-Seisdedos F, Cosset FL, Altmeyer R. C-  
45 type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype  
46 particles. *J Biol Chem* 2004;279:32035-45.  
47  
48 86. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa  
49 D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998;282:938-41.  
50  
51 87. Clarke D, Griffin S, Beales L, Gelais CS, Burgess S, Harris M, Rowlands D. Evidence for the  
52 formation of a heptameric ion channel complex by the hepatitis C virus p7 protein in vitro. *J Biol*  
53 *Chem* 2006;281:37057-68.  
54  
55 88. Steinmann E, Whitfield T, Kallis S, Dwek RA, Zitzmann N, Pietschmann T, Bartenschlager R.  
56 Antiviral effects of amantadine and iminosugar derivatives against hepatitis C virus. *Hepatology*  
57 2007;46:330-8.  
58  
59  
60

- 1
- 2
- 3 89. Steinmann E, Penin F, Kallis S, Patel AH, Bartenschlager R, Pietschmann T. Hepatitis C virus p7
- 4 protein is crucial for assembly and release of infectious virions. PLoS Pathog 2007;3:e103.
- 5
- 6 90. Lorenz IC, Marcotrigiano J, Dentzer TG, Rice CM. Structure of the catalytic domain of the
- 7 hepatitis C virus NS2-3 protease. Nature 2006;442:831-5.
- 8
- 9 91. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J. Cardif is
- 10 an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature
- 11 2005;437:1167-72.
- 12
- 13 92. Tellinghuisen TL, Marcotrigiano J, Rice CM. Structure of the zinc-binding domain of an essential
- 14 component of the hepatitis C virus replicase. Nature 2005;435:374-9.
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29
- 30
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For Peer Review

## Tables

Table 1. STAT-C compounds in the pipeline.

Drug name	Company	Target / Active drug	Study phase
<b>NS3/4A protease inhibitors</b>			
Ciluprevir (BILN 2061)	Boehringer Ingelheim	Active site / macrocyclic	Stopped
Telaprevir (VX-950)	Vertex	Active site / linear	Phase 3
Boceprevir (SCH503034)	Schering-Plough	Active site / linear	Phase 3
TMC435350	Tibotec / Medavir	Active site / macrocyclic	Phase 2
R7227 / ITMN-191	InterMune / Roche	Active site / macrocyclic	Phase 2
MK-7009	Merck	Active site / macrocyclic	Phase 2
BI201335	Boehringer Ingelheim	Active site / macrocyclic?	Phase 2
Narlaprevir (SCH900518)	Schering-Plough	Active site / linear	On hold
BMS-650032	Bristol-Myers Squibb	Active site	Phase 1
PHX1766	Phenomix	Active site	Phase 1
ACH-1625	Achillion	Active site / macrocyclic?	Phase 1
<b>Nucleoside analogue NS5B polymerase inhibitors</b>			
Valopicitabine (NM283)	Idenix/ Novartis	Active site / NM107	Stopped
R7128	Roche / Pharmasset	Active site / PSI-6130	Phase 2
R1626	Roche	Active site / R1479	Stopped
PSI-7851	Pharmasset	Active site	Phase 1
IDX184	Idenix	Active site	Phase 1
<b>Non-nucleoside NS5B polymerase inhibitors (NNI)</b>			
BILB 1941	Boehringer Ingelheim	NNI site 1 / thumb 1	Stopped
BI207127	Boehringer Ingelheim	NNI site 1 / thumb 1	Phase 2
MK-3281	Merck	NNI site 1 / thumb 1	Stopped
Filibuvir (PF-00868554)	Pfizer	NNI site 2 / thumb 2	Phase 2
VCH759	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1
VCH916	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1
VCH222	ViroChem Pharma	NNI site 2 / thumb 2	Phase 1
ANA598	Anadys	NNI site 3 / palm 1	Phase 1
HCV-796	ViroPharma / Wyeth	NNI site 4 / palm 2	Stopped
GS-9190	Gilead	NNI site 4 / palm 2	Phase 2
ABT-333	Abbott	NNI site 4 / palm 2	Phase 1
<b>NS5A inhibitor</b>			
BMS-790052	Bristol-Myers Squibb	NS5A domain 1 inhibitor	Phase 2
<b>Indirect inhibitors / unknown mechanism of action</b>			
Debio 025	Debiopharm / Novartis	Cyclophilin inhibitor	Phase 1
NIM811	Novartis	Cyclophilin inhibitor	Phase 1
SCY-635	Scynexis	Cyclophilin inhibitor	Phase 1
Nitazoxanide		PKR induction ?	Phase 2
Celgosivir	Migenix	Alpha-glucosidase inhibitor	Phase 2



Table 2: Mutations conferring resistance to telaprevir

Telaprevir-Resistant Variant	Level of Resistance (Fold change in HCV replicon IC50)
T54A	low (6)
V36M	low (7)
R155K	low (7)
A156S	low (10)
R155T/I	medium (20)
V36M+R155K	high (>60)
A156V/T	high (>62)

Table 3. Resistance mutations to HCV NS3 protease inhibitors (modified from<sup>13</sup>).

	V36A/M	T54S/A	V55A	Q80R/K	R155K/T/Q	A156S	A156T/V	D168A/V/T/H	V170A/T
Telaprevir (linear)			*						*
Boceprevir (linear)							*		
SCH900518 (linear)									
BILN-2061 (macrocylic)									
R7227/ITMN191 (macrocylic)						*	*		
MK-7009 (macrocylic)									
TMC435350 (macrocylic)									
BI-201335 (macrocylic?)									

\* mutations associated with resistance in vitro but not described in patients

## Figures legends

Figure 1. The HCV replication complex. After clathrin-mediated endocytosis, fusion of HCV with cellular membranes, and uncoating the viral nucleocapsid, the single-stranded positive-sense RNA genome of the virus of approximately 9600 nucleotides is released into the cytoplasm to serve as a messenger RNA for the HCV polyprotein precursor. The HCV genome contains a single large open reading frame encoding for a polyprotein of about 3100 amino acids. The translated section of the HCV genome is flanked by the strongly conserved HCV 3' and 5' untranslated regions (UTR). The 5' UTR is comprised of four highly structured domains forming the internal ribosome entry site (IRES), which is a virus-specific structure to initiate HCV mRNA translation. From the initially translated polyprotein, the structural HCV protein core (C) and envelope 1 and 2 (E1, E2); p7; and the six non-structural HCV proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B, are processed by both viral and host proteases.

The core protein forms the viral nucleocapsid carrying E1 and E2, which are receptors for viral attachment and host cell entry. The tetraspanin protein CD81, claudin-1, occludin, scavenger receptor class B type 1 (SR-B1), the low-density lipoprotein (LDL) receptor, glycosaminoglycans and the dendritic cell- / lymph node-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN/L-SIGN) have been identified as putative ligands for E1 and E2<sup>83-86</sup>. The non-structural proteins are mainly enzymes essential for the HCV life cycle. P7 is a small hydrophobic protein that oligomerises into a circular hexamer, most likely serving as an ion channel through the viral lipid membrane<sup>7, 87-91</sup>.

NS2 and NS3 are viral proteases required for the procession of the HCV polyprotein. NS2 is a metalloproteinase that cleaves itself from the NS2/NS3 protein, leading to its own loss of function and to the release of the NS3 protein<sup>7, 90, 91</sup>. NS3 provides a serine protease activity and a helicase/NTPase activity. The serine protease domain comprises two  $\beta$ -barrels and four  $\alpha$ -helices. The serine protease catalytic triad – histidine 57, asparagine 81 and serine 139 – is located in a small groove between the two  $\beta$ -barrels. NS3 forms a tight, non-covalent complex

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2  
3 with its obligatory cofactor and enhancer NS4A, which is essential for proper protein folding. The  
4  
5 NS3/4A protease cleaves the junctions between NS3/NS4A, NS4A/NS4B, NS4B/NS5A and  
6  
7 NS5A/NS5B. Besides its essential role in protein processing, NS3 is integrated into the HCV  
8  
9 RNA replication complex, supporting the unwinding of viral RNA by its helicase activity.

10  
11 NS4B and NS5B are involved in the organization of the HCV replication complex by interactions  
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13 with lipid membranes, which lead to the formation of the so called membranous web<sup>11, 12, 69, 92</sup>.

14  
15 The membranous web comprises of rearranged intracellular lipid membranes derived from the  
16  
17 endoplasmic reticulum. It provides the basis for the highly structured association of viral proteins  
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19 and RNA, and of cellular proteins and cofactors within the replication complex. In addition, NS4B  
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21 and NS5B are involved in transport of viral RNA within the replication complex<sup>11, 12, 69, 92</sup>.

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23 NS5B is an RNA-dependent RNA-polymerase which catalyzes the synthesis of a  
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25 complementary negative-strand RNA by using the positive-strand RNA genome as a template<sup>11,</sup>  
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From this newly synthesized negative-strand RNA, numerous RNA strands of positive  
polarity are produced by NS5B activity which serve as templates for further replication and  
polyprotein translation. Because of its poor fidelity leading to a high rate of errors in its RNA  
sequencing, numerous different isolates are generated during HCV replication in a given patient,  
termed HCV quasispecies. It is thought that due to the lack of proof-reading of the NS5B  
polymerase together with the high replication rate of HCV every possible mutation will be  
generated each day. Thus, NS5B is one key factor in the development of viral resistance during  
STAT-C therapies.

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3 Figure 2. Results of PROVE 1 (USA). Combination therapy of telaprevir (TVR) and pegIFN- $\alpha$ 2a  
4 + ribavirin in treatment-naive genotype 1 patients.  
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10 Figure 3. Results of PROVE 2 (Europe). Combination therapy of telaprevir (TVR) and pegIFN-  
11  $\alpha$ 2a +/- ribavirin in treatment-naive genotype 1 patients.  
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16 Figure 4. Results of PROVE 3. Combination therapy of telaprevir (TVR) and pegIFN- $\alpha$ 2a +/-  
17 ribavirin in HCV genotype 1 patients with prior non-response or relapse to standard treatment.  
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22 Figure 5. Results of SPRINT-1. Combination therapy of boceprevir and pegIFN  $\alpha$ 2b + ribavirin  
23 (RBV) in treatment-naive genotype 1 patients.  
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28 Figure 6. Antiviral activity of NS3/4A protease inhibitors during monotherapy for 3-14 days  
29 (modified from<sup>13</sup>).  
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34 Figure 7. Antiviral activity of nucleoside analogue NS5B polymerase inhibitors during  
35 monotherapy for 3-14 days (modified from<sup>13</sup>).  
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40 Figure 8. Antiviral activity of non-nucleoside analogue NS5B polymerase inhibitors during  
41 monotherapy for 3-14 days (modified from<sup>13</sup>).  
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Figures.

Figure 1.

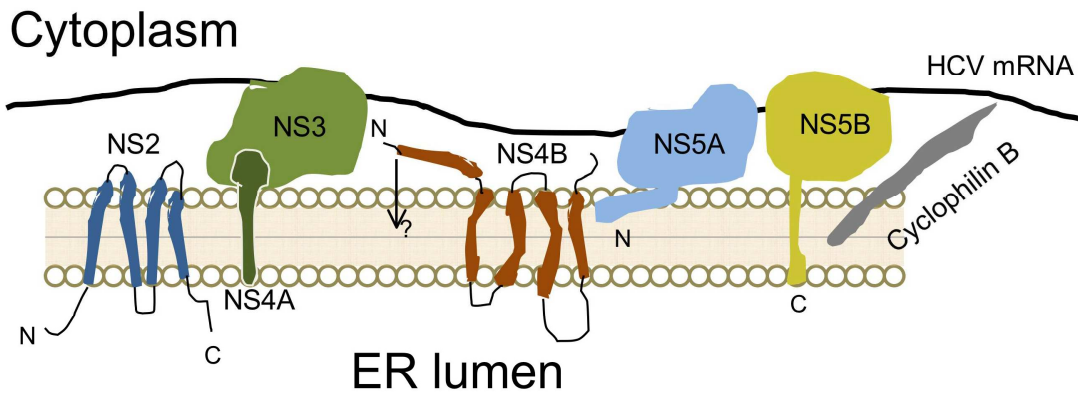


Figure 2.

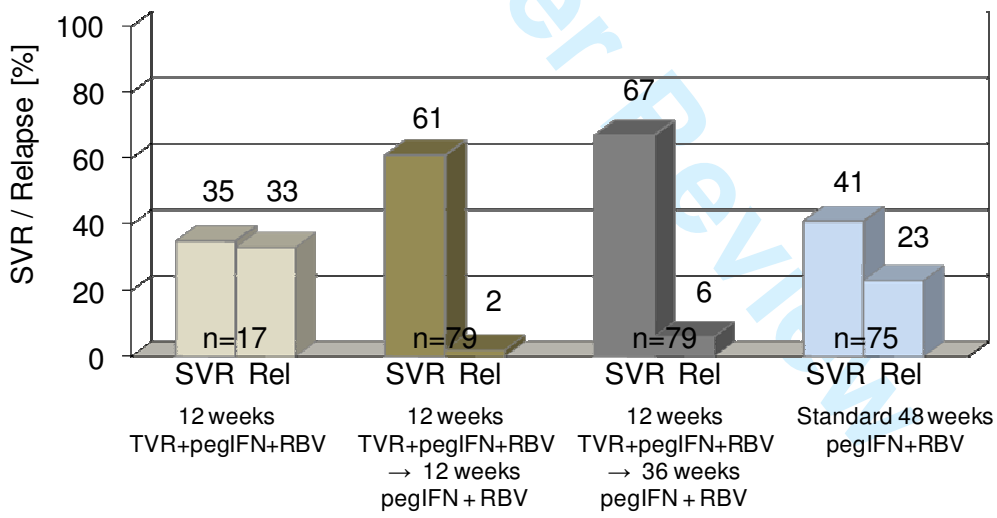


Figure 3.

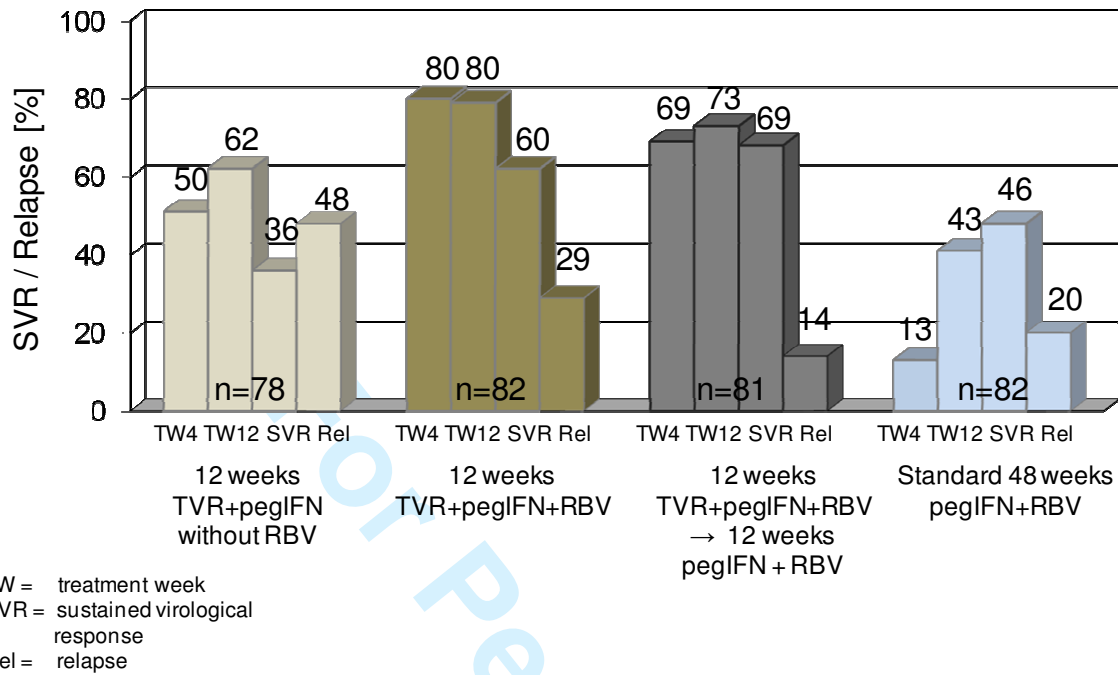


Figure 4.

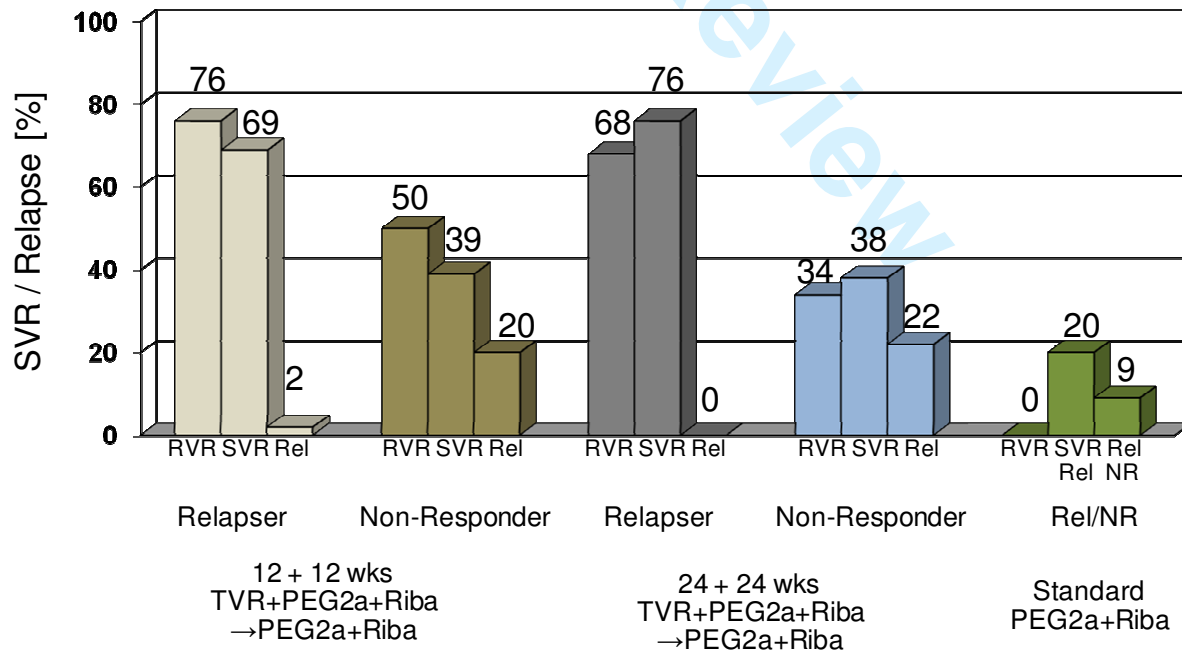


Figure 5.

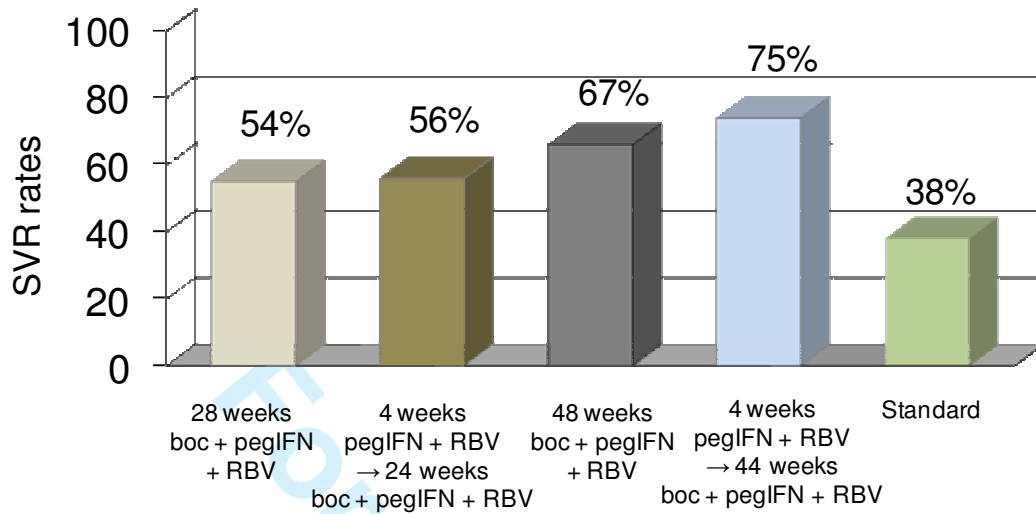


Figure 6.

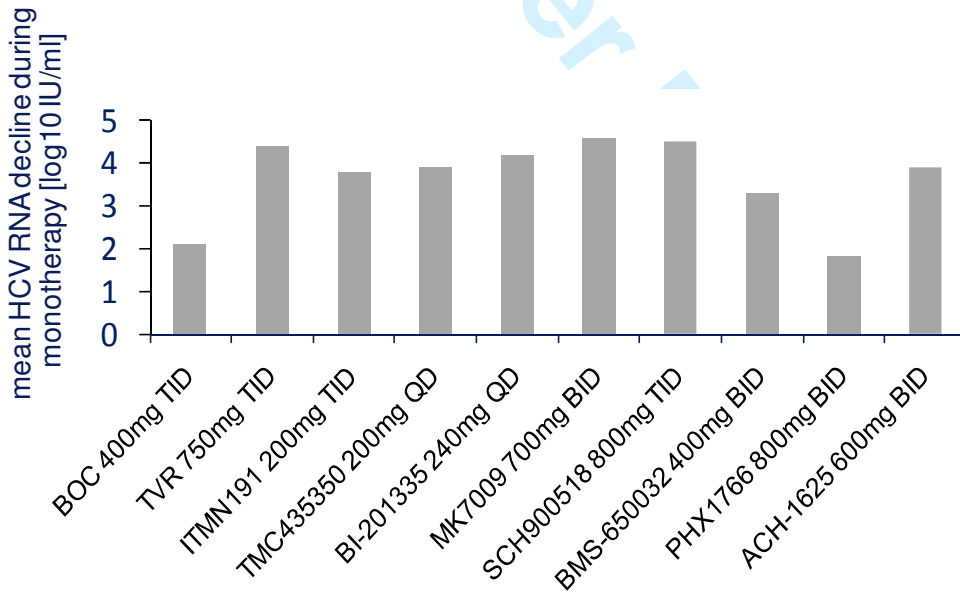


Figure 7.

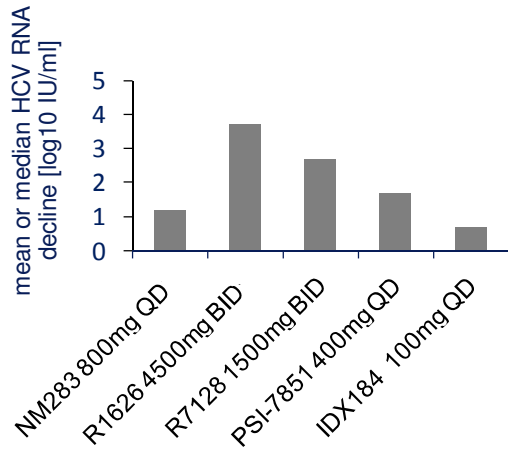
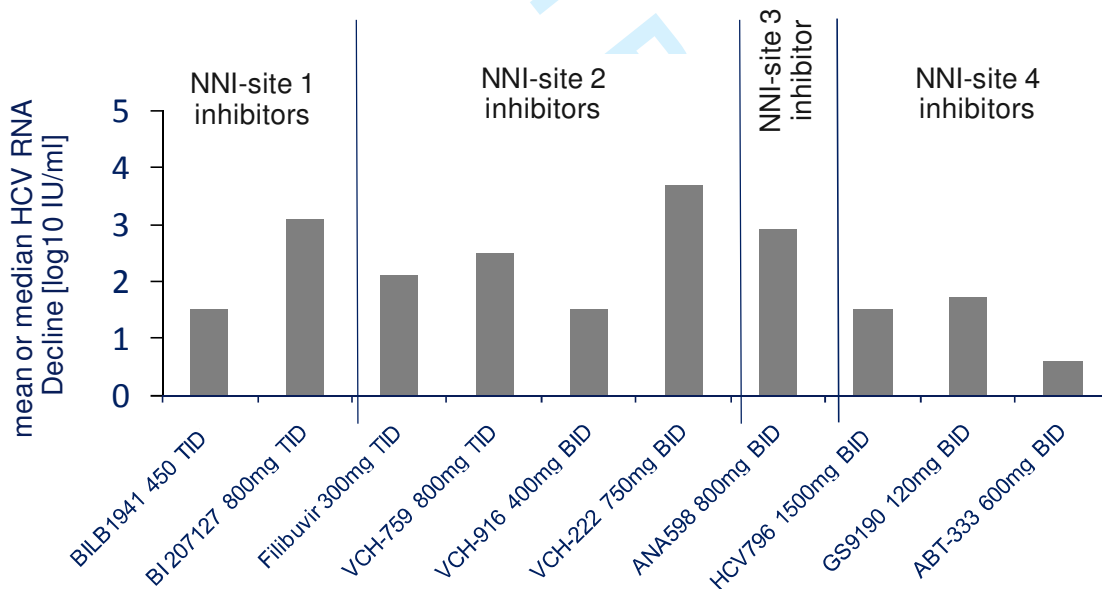
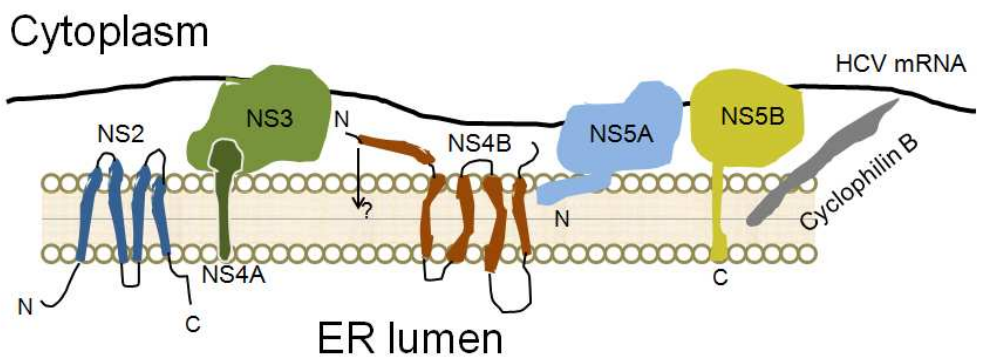


Figure 8.





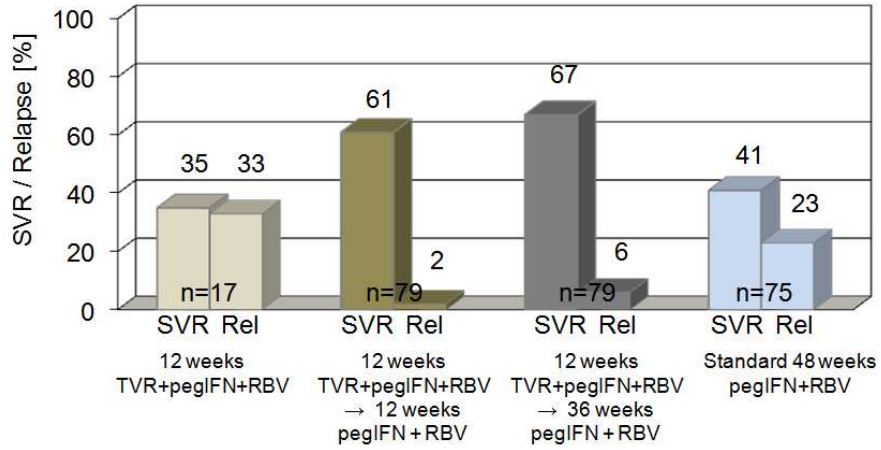
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72x27mm (300 x 300 DPI)

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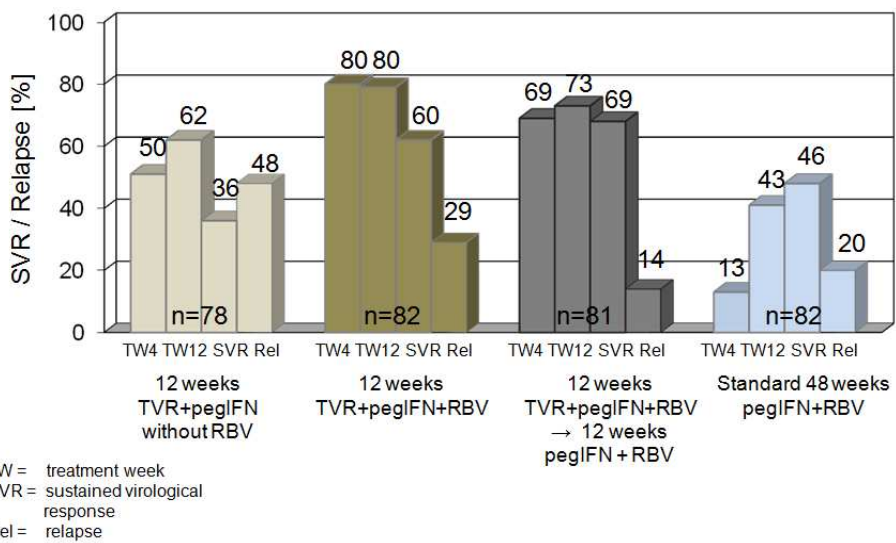
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78x37mm (300 x 300 DPI)

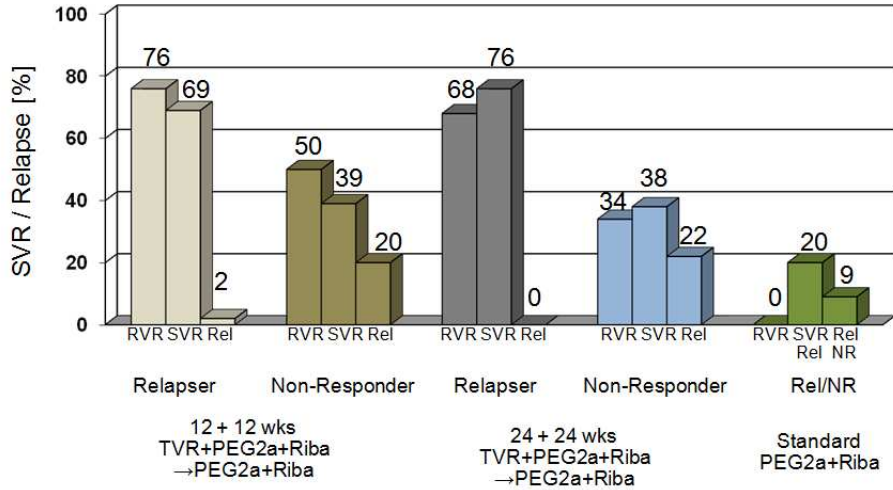
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330x184mm (72 x 72 DPI)

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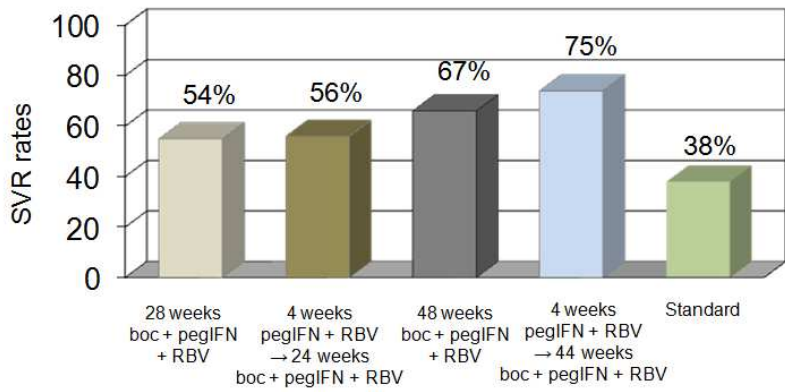


79x44mm (300 x 300 DPI)

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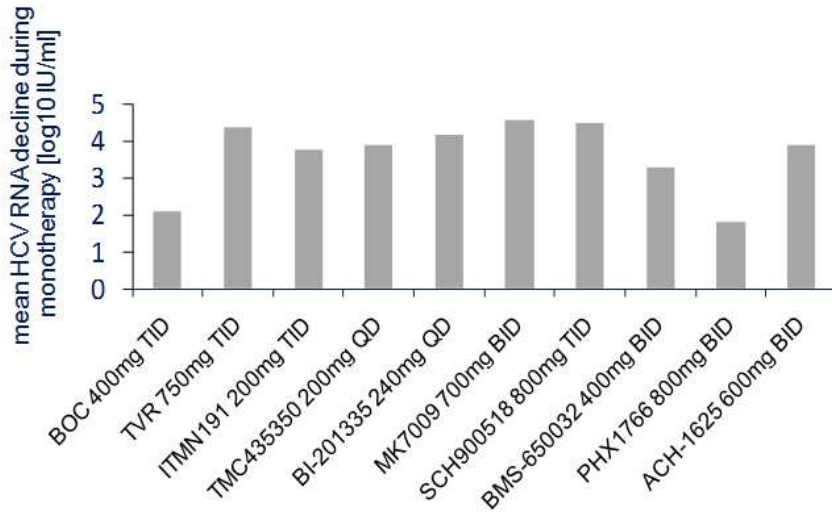
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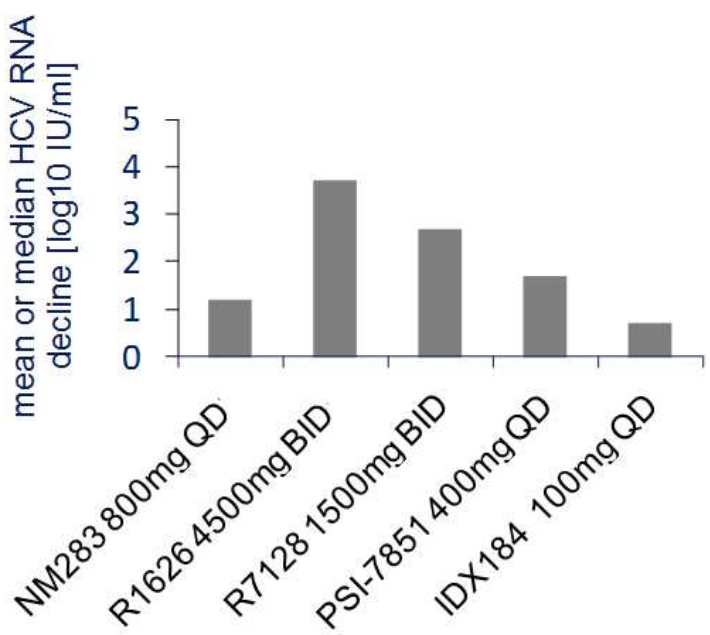
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64x37mm (300 x 300 DPI)

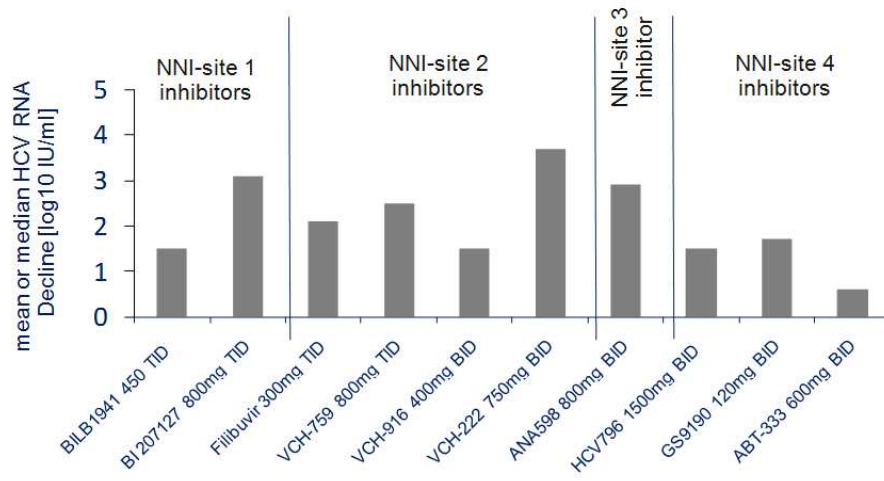
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59x45mm (300 x 300 DPI)

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79x41mm (300 x 300 DPI)

Peer Review