Review

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Perspectives and challenges of interferon-free therapy for chronic hepatitis C

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Summary

Recent data have clearly shown that a sustained virologic response can be achieved in different HCV infected patient populations with various interferon-free treatment regimens. Despite the successful implementation of telaprevir- and boceprevir-based triple therapies, all-oral regimens will certainly become a first choice for a number of HCV-infected patients in the very near future, as triple therapy approaches are burdened with significant side-effects and limited success in patients with advanced liver fibrosis and prior null-response to pegylated interferon-α (pegIFN-α)/ribavirin therapy. However, available data from phase I and II clinical trials evaluating interferon-free regimens have not yet revealed a clearly outstanding all-oral combination, and numerous challenges remain to be addressed by intensive ongoing and future research. In particular, thus far evaluated all-oral regimens did not cure a satisfactory percentage of patients with unfavorable baseline characteristics, namely patients infected with HCV genotype 1a, previous null-response to pegIFN-α/ribavirin, or liver cirrhosis. In this review, we summarize available data of interferon-free regimens for the treatment of chronic hepatitis C and assess implications for perspectives and challenges in the further development of all-oral therapies.

A need for interferon-free treatment regimens for chronic hepatitis C

The approval of the hepatitis C virus (HCV) protease inhibitors telaprevir and boceprevir in 2011 represents a major breakthrough in the treatment of chronic hepatitis C. For HCV genotype 1 patients, telaprevir- or boceprevir-based combination therapy with pegylated interferon-α (pegIFN-α) and ribavirin constitutes the novel standard of care, since significantly higher SVR rates compared to pegIFN-α and ribavirin alone have been demonstrated for both treatment-naïve and -experienced patients [1–4]. Nevertheless, telaprevir or boceprevir-based triple therapy has certain limitations. In particular, the interferon-sensitivity of individual patients remains a major determinant of treatment success because a slow decline of HCV viral load during triple therapy is associated with a high risk for the selection of resistance associated variants (RAVs) [5]. Consequently, viral breakthrough of drug resistant variants was observed in a significant number of patients with partial- or null-response to previous treatment with pegIFN-α and ribavirin, in patients with limited decline of HCV viral load during lead-in treatment with pegIFN-α and ribavirin alone, or in difficult-to-cure populations like African-Americans or patients with advanced liver fibrosis [1,4]. To overcome the risk of treatment failure in such patients, triple therapy regimens, including more potent directly acting antiviral agents (DAA), or quadruple therapies based on therapy of pegIFN-α and ribavirin plus combination of two DAAs derived from different molecular classes, may be applicable. A high potential of these approaches has already been demonstrated in phase I and II clinical trials, with outstanding SVR rates especially after quadruple therapy even in previous null responders to pegIFN-α and ribavirin alone [6,7]. However, these clinical trials were performed in highly selected patients, and both triple and quadruple therapy approaches are no option for patients with contraindications to pegIFN-α or ribavirin, such as patients with decompensated liver cirrhosis or liver transplant failure. This is especially relevant in view of the rising age of the HCV-infected population in the Western world, which implicates an increasing number of patients with advanced liver disease and previous treatment failure in the next decade [8]. Hence, a large count of patients with chronic hepatitis C not tolerating IFN-α but urgently requiring antiviral therapy can be anticipated in the near future. To be forearmed to this significant medical need – and to offer "easier-to-treat" patients, more convenient treatment modalities than IFN-α-based regimens – intensive research currently addresses the potential of interferon-free, all-oral DAA therapies. In this review, we summarize available safety and efficacy data of these interferon-free regimens and offer an assessment of future perspectives and limitations of all-oral therapies.

Keywords: Hepatitis C virus; Antiviral therapy; Directly acting antiviral agent; All-oral therapy; Null responder.
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Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; SVR, sustained virologic response; RVR, rapid virologic response; eRVR, extended rapid virologic response; EVR, early virologic response; peg, pegylated; IFN, interferon; *IL28B*, interleukin 28B; DAA, directly acting antiviral agent.

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Key Points

- The increasing number of HCV-infected patients with advanced liver disease and patients with contraindications to interferon-based therapies represents an urgent medical need to develop potent interferon-free treatment regimens
- Currently available DAAs differ significantly in their antiviral efficacy, genetic barrier to resistance, and HCV genotype coverage. Novel nucleoside analogue NS5B inhibitors (NIs) are attractive candidates for the backbone of interferon-free regimens, as they display a high antiviral activity, together with broad genotypic coverage and a high barrier to resistance. NS3-4A inhibitors and NS5A inhibitors are also characterized by a profound antiviral potency, but their barrier to resistance is relatively low
- Various combinations of two or more DAAs with or without ribavirin led to SVR in different patient populations. Powerful interferon-free regimens - at least in patients with favorable baseline characteristics - included for example the combination of the NI sofosbuvir (GS-7977) with the NS5A inhibitor daclatasvir, or combinations of NS3-4A inhibitors with NS5A inhibitors, NIs or selected non-nucleoside NS5B inhibitors, + ribavirin
- The addition of ribavirin to all-oral regimens in general had an important impact on the prevention of viral breakthrough. In selected regimens with both a high genetic barrier to resistance and potent antiviral activity, the addition of ribavirin may be unnecessary
- Previous non-response to PegIFNα and ribavirin therapy, infection with HCV subtype 1a, poor compliance, and poor-response *IL28B* genotype are predictors of failure of interferon-free treatment regimens. However, the relevance of these negative predictors of treatment outcome differs significantly according to the potency of specific all-oral regimens
- A better characterization of HCV quasispecies at baseline and after failure of interferon-free regimens is necessary to clarify the impact of resistance-associated variants (RAVs) on outcome and choice of specific all-oral regimens
- A case of late relapse between week 24 and 36 after completion of treatment with ABT-450/r, ABT-072, and ribavirin may indicate a need for longer follow-up times than SVR_{24} after treatment with all-oral regimens

The current repertoire of DAA agents for all-oral combination therapies

HCV NS3-4A protease inhibitors

NS3-4A inhibitors target the shallow enzymatic groove of the HCV protease and thereby inhibit HCV polyprotein procession, a crucial step in the early HCV life cycle [9]. In the meanwhile, numerous NS3-4A protease inhibitors have been developed which can be divided into two molecular classes, the macrocyclic inhibitors and linear tetra-peptide α-ketoamide derivatives [9] (Table 1). In general, NS3-4A inhibitors are characterized by a remarkable antiviral activity, but also by a low barrier to resistance. Hence, as it was shown for example for the approved α-ketoamide derivatives telaprevir and boceprevir, monotherapy with NS3-4A inhibitors results in an approximately 4 $\log_{10}$ decrease of serum HCV RNA within days, but also in a rapid selection of resistant variants and viral breakthrough [10–13]. The risk of resistance development can be significantly reduced by the addition of pegIFN-α and ribavirin, and telaprevir or boceprevir-based triple therapies result in SVR rates of approximately 70–80%, 80–90%, and 30–40% in treatment-naïve HCV genotype 1 patients, previous relapsers, and null responders to pegIFN-α and ribavirin, respectively [1–4].

Another important feature of most NS3-4A protease inhibitors is the selective activity against distinct HCV genotypes, which is explained by sequence differences in important parts of the protease domain between HCV genotypes [5]. Thus far, most NS3-4A inhibitors have been developed predominantly to target HCV genotype 1. Newer NS3-4A protease inhibitors than telaprevir and boceprevir, which are currently in phase 1–3 development, include for example simeprevir (TMC435), danoprevir (R7227/ITMN191), vaniprevir (MK-7009), asunaprevir (BMS-650032), BI201335, ACH-1625, ABT-450, MK-5172, GS-9256, and GS-9451. Potential advantages of these second and third generation protease inhibitors might be improved tolerability, broader genotypic activity (e.g., MK-5172), different resistance profiles (e.g., MK-5172), and/or improved pharmacokinetics, which allow a once daily dosage (e.g., TMC435, BI201335) [14–18].

Unfortunately, the resistance profiles of linear tetrapeptide and macrocyclic inhibitors are overlapping. Amino acid position R155 in NS3 constitutes the central position for resistance development [19]. Mutations at this amino acid site confer resistance to nearly all protease inhibitors which are currently in advanced clinical development. Consequently, combining different NS3-4A inhibitors is not a logical strategy for interferon-free regimens. A possible exception is MK-5172, which exhibits potent antiviral activity against variants carrying mutations at position R155 [16].

Importantly, the genetic barrier to resistance against telaprevir (and other NS3-4A inhibitors) differs significantly between HCV genotype 1 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 HCV genotype 1b [2,4]. This difference was shown to result from nucleotide differences at position 155 in HCV subtype 1a (AGA, encodes R) *vs.* 1b (CGA, also encodes R). The mutation most frequently associated with resistance to telaprevir is R155K; changing R to K at position 155 requires 1 nucleotide change in HCV subtype 1a and 2 nucleotide changes in subtype 1b isolates [20]. Consequently, HCV genotype 1a may be a problematic subtype for successful all-oral therapy based on NS3-4A inhibitors.

An additional possible limitation of most NS3-4A inhibitors is the interaction with CYP3A4, resulting in numerous drug–drug interactions including tacrolimus, cyclosporine, antiretroviral agents, statins, antifungals, and many more [21]. This complicates their use in distinct patient populations with a high need for interferon-free regimens, such as liver transplanted patients or patients co-infected with human immunodeficiency virus (HIV).

Table 1. Selected DAAs and host-targeting agents (HTAs) in the pipeline.

Drug name	Company	Target/active site	Phase
NS3-4A protease inhibitors			
Ciluprevir (BILN 2061)	Boehringer Ingelheim	Active site/macrocyclic	Stopped
Telaprevir (VX-950)	Vertex	Active site/linear	IV
Boceprevir (SCH503034)	Schering-Plough	Active site/linear	IV
Simeprevir (TMC435)	Janssen/Medivir	Active site/macrocyclic	III
Danoprevir (R7227)	Roche/InterMune	Active site/macrocyclic	III
Vaniprevir (MK-7009)	Merck	Active site/macrocyclic	Halted/II
MK-5172	Merck	Active site/macrocyclic	II
BI201335	Boehringer Ingelheim	Active site/linear	III
Narlaprevir (SCH900518)	Schering-Plough	Active site/linear	Halted
Asunaprevir (BMS-650032)	Bristol-Myers Squibb	Active site	II
PHX1766	Pheromix	Active site	Halted
GS-9256	Gilead	Active site	II
GS-9451	Gilead	Active site	I
ABT450	Abbott	Active site	II
IDX320	Idenix	Active site	II
ACH-1625	Achillion	Active site/macrocyclic?	II
Nucleoside analogue NS5B polymerase inhibitors			
Valopicitabine (NM283)	Idenix/Novartis	Active site	Stopped
Mericitabine (R7128)	Roche/Pharmasset	Active site	III
R1626	Roche	Active site	Stopped
Sofosbuvir (GS-7977) (former PSI-7977)	Pharmasset	Active site	III
PSI-938	Pharmasset	Active site	Stopped
IDX184	Idenix	Active site	Halted
ALS-220	Alios/Vertex	Active site	I
Non-nucleoside NS5B polymerase inhibitors (NNI)			
BILB 1941	Boehringer Ingelheim	NNI site 1/thumb 1	Stopped
BI207127	Boehringer Ingelheim	NNI site 1/thumb 1	II
MK-3281	Merck	NNI site 1/thumb 1	Stopped
TMC647055	Janssen	NNI site 1/thumb 1	I
Filibuvir (PF-00868554)	Pfizer	NNI site 2/thumb 2	II
VX-759	Vertex	NNI site 2/thumb 2	Halted
VX-916	Vertex	NNI site 2/thumb 2	Halted
VX-222	Vertex	NNI site 2/thumb 2	II
Setrobuvir (ANA598)	Anadys	NNI site 3/palm 1	II
ABT-072	Abbott	NNI site 3/palm 1	Halted
ABT-333	Abbott	NNI site 3/palm 1	II
HCV-796	ViroPharma/Wyeth	NNI site 4/palm 2	Stopped
Tegobuvir (GS-9190)	Gilead	NNI site 4/palm 2	II
IDX375	Idenix	NNI site 4/palm 2	II
NS5A inhibitors			
Daclatasvir (BMS-790052)	Bristol-Myers Squibb	NS5A domain 1 inhibitor	III
BMS-824393	Bristol-Myers Squibb	NS5A protein	I
PPI-461	Presidio Pharmaceuticals	NS5A protein	I
GS-5885	Gilead	NS5A protein	II
ABT-267	Abbott	NS5A protein	II
MK-8742	Merck	NS5A protein	I
Drugs targeting host factors			
Alisporivir (Debio-025)	Novartis	Cyclophilin inhibitor	Halted
NIM811	Novartis	Cyclophilin inhibitor	Halted
SCY-635	Scynexis	Cyclophilin inhibitor	II
Miravirsen	Santaris	miRNA122 antisense RNA	II

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In summary, the high antiviral activity of NS3-4A protease inhibitors predestines these agents as favorable partners for all-oral regimens. However, as discussed below in detail, adequate combination partners are required to overcome the high risk for resistance development against NS3-4A inhibitors, and their usage in patients infected with HCV genotypes 2–6 is still limited.

HCV NS5A inhibitors

The HCV NS5A protein plays a manifold role in HCV replication, assembly and release [22]. Hence, pharmacological inhibition of NS5A can tackle the HCV life cycle effectively at various stages. It was shown that doses of NS5A inhibitors in a picomolar range can sufficiently suppress HCV replication *in vitro* [23]. Furthermore, due to conserved structural features of domain I of NS5A, currently developed NS5A inhibitors are highly effective against all HCV genotypes. Despite these conserved structural features, NS5A inhibitors display a low genetic barrier to resistance, which resulted in rapid selection of RAVs during monotherapy [24].

Daclatasvir (BMS-790052) was the first NS5A inhibitor reaching clinical evaluation. Daclatasvir monotherapy resulted in an approximately 4 $\log_{10}$ HCV RNA decline [24]. Like for NS3-4A protease inhibitors, RAVs during treatment with daclatasvir monotherapy were observed more frequently in HCV genotype 1a patients (main residues M28, Q30, L31, and Y93 of NS5A) compared to HCV genotype 1b patients (main residues L31 and Y93) [24,25]. In a phase IIa clinical trial in treatment-naïve HCV genotype 1 patients, Daclatasvir-based triple therapy resulted in extended rapid virologic response (eRVR) in up to 83% of patients, compared to 9% in the control group treated with pegIFN-α and ribavirin only [26]. Other NS5A inhibitors (e.g., BMS-824393, ABT-267, PPI-461, GS-5885) are in earlier clinical development.

Due to their high antiviral activity against all HCV genotypes, NS5A inhibitors are highly promising combination therapy partners for interferon-free therapy regimens with broad genotypic coverage. Furthermore, thus far available data indicate a good tolerability of NS5A inhibitors. However, like for NS3-4A protease inhibitors, adequate combination partners are required to overcome the low barrier to resistance against NS5A inhibitors.

HCV N5B polymerase inhibitors

NS5B RNA polymerase inhibitors can be divided into two distinct categories. Nucleoside analog inhibitors (NIs), like mericitabine (R7128) or sofosbuvir (GS-7977, former PSI-7977), mimic the natural substrates of the polymerase and are incorporated into the growing RNA chain, thus causing direct chain termination by tackling the active site of NS5B [27]. Because the active centre of NS5B is a highly conserved region of the HCV genome, NIs are usually effective against different HCV genotypes [5]. Single amino acid substitutions in every position of the active centre may result in loss of function or in extremely impaired replicative fitness. Thus, there is a high barrier to resistances for NIs.

The development of several NIs (e.g., valopicitabine, R1626, PSI-938, BMS-986094) has been stopped due to toxicity issues. Of the earlier NIs, mericitabine (RG7128) is still in clinical development. Like most early NIs, mericitabine has a moderate antiviral activity and thus far RAVs against mericitabine have been observed very rarely in clinical studies [28–30]. Mericitabine-based triple therapy in HCV genotype 1, 2, and 3 infected patients revealed superior SVR rates compared to peg-IFN-α and ribavirin alone, though the increased chance of cure was lower as compared for example to NS3-4A inhibitor-based triple therapies [31]. Very promising clinical data have been recently published for sofosbuvir (GS-7977), another NI effective against all HCV genotypes. In HCV genotype 1, 2, and 3 infected patients, short durations of sofosbuvir-based triple therapy resulted in SVR rates of 90–100% [32,33]. These data indicate that some newer NIs like sofosbuvir are also characterized by high antiviral activities. In addition, their high barrier to resistance development suggests that they are optimal candidates for interferon-free combination therapies.

In contrast to NIs, the heterogeneous class of non-nucleoside inhibitors (NNIs) achieves NS5B inhibition by binding to different allosteric enzyme sites, which results in a conformational protein change before the elongation complex is formed [34]. For allosteric NS5B inhibition, high chemical affinity is required. NS5B is structurally organized in a characteristic "right hand motif", containing finger, palm and thumb domains, and offers at least four NNI-binding sites, a benzimidazole-(thumb 1)-, thiophene-(thumb 2)-, benzothiadiazine-(palm 1)- and benzofuran-(palm 2)-binding site [35]. Because NNIs bind distantly to the active centre of NS5B, their barrier to resistance is significantly lower as for NIs, and viral breakthrough has frequently been observed in monotherapy studies evaluating NNIs [36,37]. Currently, numerous non-nucleoside inhibitors are in phase I and II clinical evaluation (e. g., thumb 1 inhibitor BI207127; thumb 2 inhibitors filibuvir (PF-00868554), and VX-222; palm I inhibitors ANA598 and ABT-333; Palm II inhibitors tegobuvir (GS-9256) and IDX-375). In general, these non-nucleoside analogues display a low to medium antiviral activity as well as a low barrier to resistance [38–41]. In contrast to NIs, NNIs in general do not display antiviral activity against different HCV genotypes [42]. In view of these characteristics, NNIs will probably not be developed as triple therapy but rather as components of quadruple or all-oral regimens (see below).

Drugs targeting host factors

HCV depends on various host factors such as cyclophilin A throughout its life cycle [43]. Alisporivir (former Debio-025) is an orally bioavailable cyclophilin A inhibitor exerting an antiviral impact on both HCV and HIV replication. Alisporivir is characterized by a relatively high antiviral activity against all HCV genotypes and by a high barrier to resistance development [44]. In treatment-naïve HCV genotype 1 patients, combination therapy with alisporivir, pegIFN-α-2a and ribavirin for 24–48 weeks resulted in SVR rates of 69–76% compared to 55% in the control group [45,46]. Despite these promising data, the development of alisporivir is currently on hold due to rare cases of severe pancreatitis during combination therapy with alisporivir and pegIFN-α-2a. During alisporivir monotherapy, pancreatitis was not observed. Nevertheless, the pan-genotypic activity of alisporivir together with the low risk of alisporivir-resistance has proven the high potential of drugs targeting host factors for interferon-free treatment regimens.

Other compounds

Numerous additional approaches to inhibit the HCV life cycle are in preclinical or early clinical development. These include viral

entry or assembly inhibitors, NS4B inhibitors, the micro-RNA-122 inhibitor miravirsen, silibinin (a flavonolignans of milk thistle, targeting the HCV life cycle at various steps), or immune-modulatory agents like toll-like receptor agonists [5,47,48]. Notably, the development of miravirsen has proven for the first time that targeting a micro-RNA might be a suitable therapeutic option for viral (or other) diseases [48]. A possible role of these agents in the treatment of chronic hepatitis C remains to be defined.

Clinical trials evaluating interferon-free treatment regimens

As it is well established for the treatment of HIV infection, combining DAA agents with different antiviral resistance profiles should result in a substantially decreased risk of viral breakthrough of RAVs [19]. Nucleoside analogue NS5B inhibitors, but also drugs targeting host factors such as the cyclophilin inhibitor alisporivir, display a high barrier to resistance and may therefore be key agents for effective DAA combination therapies. In contrast, NS3-4A and NS5A inhibitors display a low barrier to resistance development, but in view of their high antiviral efficacy they appear to be promising combination partners for NIs or cyclophilin inhibitors. Due to their low antiviral efficacy and low barrier to resistance development, the benefit of using non-nucleoside analogue NS5B inhibitors appears to be less pronounced compared to the above indicated drug classes. As described in the following, an increasing number of studies evaluating interferon-free treatment regimens have generally proven these theoretical considerations, but also yielded surprising results, e.g., with respect to the possible role of some NNIs (Table 2).

Combinations of NS3-4A inhibitors and nucleoside analogue NS5B inhibitors, with or without ribavirin

The first interferon-free clinical trial (INFORM-1 study) evaluated the combination of an NI (mericitabine, R7128) and an NS3-4A inhibitor (danoprevir, R7227). In this proof-of-principle study, patients were treated with both compounds for up to 2 weeks [49]. HCV RNA concentrations decreased up to 5.2 $\log_{10}$IU/ml, viral breakthrough was observed in only one patient (but no RAVs were identified), and HCV RNA was undetectable at the end of dosing, in up to 63% of treatment-naïve patients. However, the fundamental question whether an SVR can be achieved with combination therapies of different DAA compounds without pegIFN-α and ribavirin had to be answered by subsequent trials. In the meanwhile, the INFORM-SVR study provided SVR data for the combination of mericitabine and danoprevir (ritonavir-boosted) with or without ribavirin for 12–24 weeks [30]. SVR rates in HCV genotype 1a and 1b patients were 26% and 71% in treatment arms including ribavirin (total n = 83), respectively, but significantly lower in all ribavirin-free treatment groups (total n = 86). Importantly, RAVs in patients with viral breakthrough were predominantly identified within NS3-4A while a resistance mutation in NS5B was discovered only in a single patient (S282T).

Several phase II clinical trials assessed the combination of the NI sofosbuvir (GS-7977) with or without ribavirin. In the QUANTUM trial, treatment-naïve HCV genotype 1, 2, and 3 patients were treated for 12–24 weeks with sofosbuvir and ribavirin. In a recent interims analysis, 59% of HCV genotype 1 patients who completed the 12-week course of therapy achieved SVR at week 4 post treatment [50]. In this preliminary analysis, *IL28B* genotype appeared to be a predictor of treatment outcome. In ELECTRON, the same 12-week regimen resulted in SVR in only 11% (1/9) of HCV genotype 1a null responders, but in 88% (22/25) of treatment-naïve HCV genotype 1 patients, and in 80% (12/15) of treatment-experienced HCV genotype 2 and 3 patients, and in 100% (10/10) of treatment-naïve HCV genotype 2 and 3 patients [51,52]. In addition, the ELECTRON study evaluated sofosbuvir monotherapy in treatment-naïve HCV genotype 2 and 3 patients, which, however, resulted in SVR in only 60% (6/10) of the cases, highlighting again the important role of ribavirin in interferon-free treatment regimens [51]. In the meanwhile, phase III approval trials (FISSION, POSITRON, FUSION, and NEUTRINO) have been initiated to evaluate sofosbuvir + ribavirin for 12–16 weeks in HCV genotype 2 and 3 patients.

Combinations of NS3-4A inhibitors and non-nucleoside analogue NS5B inhibitors, with or without ribavirin

The SOUND-C1 trial assessed the combination therapy of the NS3-4A inhibitor BI-201335, the NNI BI-207127 (400 or 600 mg q8h) and ribavirin for 4 weeks [53]. Virologic response rates in patients treated with 600 mg q8h of BI-207127 were 82%, 100%, and 100% at treatment days 15, 22, and 29, respectively. In patients who received the lower dose of BI-207127, virologic response rates were significantly lower, and in these patients lower virologic response rates were observed for patients infected with HCV subtype 1a compared to HCV subtype 1b. SOUND-C1 provided no SVR rates for this all-oral regimen, since patients received pegIFN-α-based therapy from treatment week five. However, the larger SOUND-C2 trial provided SVR rates for BI-201335 in combination with BI-207127 with or without ribavirin, administered for 16–40 weeks in approximately 360 treatment-naïve HCV genotype 1 patients [54]. Overall, SVR12 rates ranged from 56% to 68% in treatment arms including ribavirin, compared to 39% in a single ribavirin-free treatment arm. Within all treatment arms, SVR rates were consistently higher in HCV genotype 1b *vs.* 1a patients or in patients with a good-response *IL28B* genotype.

The combination of tegobuvir (GS-9190), another NNI, with GS-9256, an NS3-4A inhibitor, ± ribavirin was assessed in another trial in treatment-naïve HCV genotype 1 patients [55]. Importantly, tegobuvir + GS-9256 + ribavirin led to a higher RVR rates compared to tegobuvir + GS-9256 alone (38% *vs.* 7%, respectively), further proving the possible benefit of ribavirin in distinct interferon-free DAA combination therapies. However, with tegobuvir and GS-9256-based quadruple therapy (pegIFN-α, and ribavirin), RVR was achieved in 100% of patients.

A comparable approach is followed in the ZENITH trial, assessing the antiviral activity of the NS3-4A inhibitor telaprevir and the NNI VX-222 alone, in combination with ribavirin, or in combination with pegIFN-α and ribavirin (quadruple therapy), in treatment-naive HCV genotype 1 patients. Again, quadruple therapy led to high SVR rates. However, in the all-oral treatment arms, high rates of viral breakthrough were observed [56].

Compared to the above described data for all-oral combinations based on NS3-4A inhibitors plus NNIs, results of the recently presented Co-Pilot study were strongly encouraging. In Co-Pilot, 12 weeks of combination therapy with the NS3-4A inhibitor ABT-450 (ritonavir-boosted), the NNI ABT-333, and

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Table 2. Summary of presented interferon-free trials.

DAA combination	Study name	Patient population	Major endpoints
Nucleoside NS5B inhibitor ± ribavirin			
GS-7977 + ribavirin	Electron [51, 52], Quantum [50]	Treatment-naïve and experienced HCV Gt1-6	SVR (11-88% in HCV genotype 1 patients, dependent on previous treatment outcome, HCV subtype, *IL28B* genotype; 80-100% in HCV genotype 2/3 patients, dependent on previous treatment outcome)
GS-7977 monotherapy	Electron [51]	Treatment-naïve HCV Gt2,3	SVR (60%)
Nucleoside NS5B inhibitor + NS3-4A inhibitor ± ribavirin			
Mericitabine + danoprevir	Inform-1 [49]	Treatment-naïve HCV Gt1	HCV RNA decline at week 2 (5.2 $\log_{10}$)
Mericitabine + danoprevir/ritonavir ± ribavirin	Inform-SVR [30]	Treatment-naïve HCV Gt1	SVR (26% in HCV Gt1a, 71% in HCV Gt1b; significantly lower without ribavirin)
Nucleoside NS5B inhibitor + NS5A inhibitor ± ribavirin			
GS-7977 + daclatasvir ± ribavirin	AI-444040 [60]	Treatment-naïve HCV Gt1,2,3	SVR (100% and 91% in HCV genotype 1 and 2/3 patients, respectively)
Non-nucleoside NS5B inhibitor + NS3-4A inhibitor ± ribavirin			
BI-207127 + BI-201335 + ribavirin	SOUND-C1 [53]	Treatment-naïve HCV Gt1	RVR (73-100%, depending on dosage of BI-207127)
BI-207127 + BI-201335 ± ribavirin	SOUND-C2 [54]	Treatment-naïve HCV Gt1, including 10% of patients with compensated liver cirrhosis	SVR (56-68% + ribavirin, 39% - ribavirin; HCV Gt 1b and good-response *IL28B* genotype were additional predictors of SVR)
Tegobuvir + GS-9256 ± ribavirin	n.a. [55]	Treatment-naïve HCV Gt1	RVR (37% + ribavirin, 7% - ribavirin)*
ABT-333 + ABT-450/ritonavir + ribavirin	CO-PILOT [57]	HCV Gt1, treatment-naïve and non-responders	SVR (93% and 47% in treatment-naïve and non-responders, respectively)
ABT-072 + ABT-450/ritonavir + ribavirin	PILOT [58]	Treatment-naïve HCV Gt1 with good-response *IL28B* genotype	SVR (91% at week 24, a late relapse was observed at week 36)
VX-222 + telaprevir ± ribavirin	ZENITH [56]	Treatment-naïve HCV Gt1	SVR**
NS3-4A inhibitor + NS5A inhibitor ± ribavirin			
Asunaprevir + daclatasvir	AI447011 [6]	HCV Gt1 null responders	SVR (36%, relapse only in HCV genotype 1a but not 1b patients)*
Asunaprevir + daclatasvir	AI447017 [7]	HCV Gt1b null responders	SVR (100%)
Asunaprevir + daclatasvir	AI447017 [59]	HCV Gt1b, null responders and patients intolerant/ineligible to IFN	SVR (91% and 64% in null responders and intolerant/ineligible patients, respectively. This result highlights the importance of adherence to IFN-free regimens).
Multiple DAA agent combinations			
NS5A-inhibitor (GS-5885) + NS3-4A inhibitor (GS-9451) + NNI (tegobuvir) + ribavirin	QUAD [61]	Treatment-naïve HCV Gt1	SVR (77% and 89% in HCV genotype 1a and 1b, respectively; approx. 30% of patients required IFN-based rescue therapy due to failure of HCV RNA <LOD at week 2)
Cyclophilin-inhibitor ± ribavirin			
Alisporivir ± ribavirin	VITAL-1 [62]		SVR (approx. 90% of patients eligible for all-oral therapy, 29-42% of all patients, remaining patients received additional PegIFN-α).

*These studies include quadruple therapies resulting in 100% SVR [6] and 100% RVR [55], respectively.
**SVR rates for all-oral arms have not yet been presented for this study. Quadruple-therapy arms in ZENITH resulted in high SVR rates.

ribavirin resulted in 93% and 47% SVR in treatment-naïve HCV genotype 1 patients and in previous null responders to pegIFN-α and ribavirin alone, respectively [57]. In addition, in the single arm of the so-called Pilot study evaluating ABT-450 in combination with the NNI ABT-072 and ribavirin, SVR was achieved in 91% (10/11) of treatment-naïve HCV genotype 1 patients, who, however, all had a good-response *IL28B* genotype [58]. Importantly, a single patient in Pilot, who had achieved SVR 24 weeks after treatment completion, experienced a late viral relapse between week 24 and 36 post treatment. In this patient, a resistant mutant in NS5B has been discovered, and the question remains whether interferon-free treatment regimens require longer follow-up times than they have been established for pegIFN-α and ribavirin therapy.

Combinations of NS3-4A inhibitors and NS5A inhibitors

The first clinical trial which has reported SVR data for an interferon-free regimen investigated therapy with the NS5A inhibitor daclatasvir (BMS-790052) in combination with the NS3-4A

protease inhibitor asunaprevir (BMS-60032) for 24 weeks in 10 HCV genotype 1 patients with prior null response to pegIFN-α and ribavirin [6]. Importantly, 36% of all patients achieved SVR 24 weeks after treatment completion. All patients with viral breakthrough were infected with HCV genotype 1a, and in all of them RAVs against both agents were detected. Importantly, a quadruple arm included in this study (daclatasvir, asunaprevir, pegIFN-α, and ribavirin) resulted in 100% SVR in both HCV genotype 1a and 1b patients, highlighting the perhaps still important role of pegIFN-α and ribavirin in preventing resistance in such highly difficult-to-cure patient populations. Nevertheless, this trial constituted a proof-of-principle that SVR can be achieved by all-oral regimens, especially in patients infected with HCV subtype 1b. This has been confirmed with a 100% SVR rate by a small study evaluating the same agents (daclatasvir and asunaprevir) in Japanese HCV genotype 1b infected previous null responder patients [7], and by a subsequent Japanese study in HCV genotype 1b patients with prior null response (n = 21) or ineligibility to IFN-therapy (n = 22), in whom SVR rates of 91% and 64% have been achieved, respectively [59].

Combinations of NS5A inhibitors and nucleoside analogue NS5B inhibitors with ribavirin

Impressive results have been shown for the combination of the NS5A inhibitor daclatasvir with the NI sofosbuvir, with or without ribavirin for 24 weeks [60]. In approximately 90 treatment-naïve patients, RVR and SVR rates were 100% and 100% in HCV genotype 1 patients, and 100% and 91% in HCV genotype 2 and 3 patients, respectively. In this study, the addition of ribavirin did not improve virologic response rates but resulted in anemia in a significant proportion of patients. Furthermore, an unfavorable *IL28B* genotype apparently did not decrease the chance of cure in this study. Due to different strategies in the pipelines of Bristol-Myers Squibb and Gilead, the development of this promising regimen has been halted.

Combinations of multiple DAAs with ribavirin

A preliminary analysis of a first study evaluating a regimen containing multiple DAAs, namely NS5A inhibitor GS-5885, NS3-4A inhibitor GS-9451, NNI tegobuvir, and ribavirin has been presented recently [61]. In this so called QUAD study, treatment-naïve HCV genotype 1 patients were treated for 12–24 weeks with this all-oral quadruple regimen. Patients were switched to a pegIFN-α-based rescue therapy if HCV RNA did not fall below the limit of detection until treatment week 2. Approximately 70% of all included patients were eligible for all-oral therapy in this study, among them, at least 77% and 89% of HCV genotype 1a and 1b patients achieved SVR, respectively.

Cyclophilin inhibitor-based therapies

The VITAL-1 phase IIb clinical study evaluated the cyclophilin inhibitor alisporivir with or without ribavirin for 24 weeks in treatment-naïve HCV genotype 2 and 3, complemented by the addition of pegIFN-α if no RVR was achieved [62]. SVR rates in the per protocol analyses were approximately 90% in all treatment arms, but only 29–42% of all patients were eligible for treatment with all-oral therapy.

Perspectives and challenges for the further development of IFN-free regimens

The above described data have clearly shown that an SVR can be achieved in different HCV infected patient populations with various interferon-free DAA combination regimens. In view of the inconvenience and high rate of significant side-effects of IFN-based therapy, all-oral regimens will therefore certainly become a first choice for a number of patients in the very near future, a scenario which had appeared speculative until very recently. Nevertheless, available data from phase I and II clinical trials evaluating interferon-free regimens have not yet revealed a clearly outstanding all-oral combination, and numerous challenges remain to be addressed by intensive research.

A key lesson from the body of available data is that those patients who do not respond well to IFN-α-based therapies, especially previous null responders to pegIFN-α and ribavirin therapy, do not respond well to many interferon-free regimens as well, independently of whether these regimens include ribavirin or not. Exemplarily, as described above in detail, the combination regimen of the NS3-4A inhibitor ABT-450/r, NNI ABT-333, and ribavirin resulted in SVR rates of 100% and 47% in HCV genotype 1 treatment-naïve patients and previous null responders, respectively [57]. In addition to previous treatment outcome, *IL28B* genotype appears to be a predictor of success of some (but not of all) interferon-free regimens, although its impact is significantly attenuated compared to pegIFN-α and ribavirin dual therapy [54,61,63]. Regarding the completely different mode of operation of immune-modulating IFN-α-based therapy in contrast to IFN-free DAA regimens, which directly tackle HCV, these findings are not self-evident. A nearby explanation of these results might be that an appropriate endogenous immune response against HCV is still required for the final clearance of residual, possibly drug-resistant virus escaping DAA combinations. In addition to these host-associated determinants of treatment outcome, there clearly exist virus-associated factors which obviously attain significant importance in the upcoming era of interferon-free therapy. For all-oral regimens (and for IFN-α-based triple therapy approaches) based on either NS3-4A inhibitors or NS5A inhibitors, lower SVR rates have consistently been observed in HCV genotype 1a *vs.* 1b patients, a difference which can be explained by the significantly lower genetic barrier of resistance mutations against these drugs at defined positions in the HCV genotype 1a *vs.* 1b genome [19,20,25]. Hence, HCV genotype 1a infected patients will require special attention in the era of DAA therapies.

The high replication rate of HCV and the poor fidelity of its RNA-dependent RNA polymerase are reasons for numerous HCV variants (quasispecies) which are continuously produced during HCV replication [19]. Among them, variants carrying mutations altering the conformation of the binding sites of DAA compounds can develop. Whether such naturally existing HCV isolates, which may confer resistance to DAAs, have an impact on the success of IFN-free combination therapy is currently not well defined [64,65]. However, it appears plausible that such pre-existing HCV variants, which have been vastly neglected during the era of pegIFN-α and ribavirin therapy, can significantly influence the outcome of IFN-free treatment regimens [64,65]. Intensive research is required to clarify whether the presence of distinct HCV isolates at baseline may already predict a limited chance

of cure with specific DAA combinations in a given patient. Furthermore, a detailed characterization of the HCV quasispecies in patients who failed to be cured by IFN-free therapies will be important, in particular with respect to the selection of appropriate second-line regimens.

How can these insights be translated into the development of an ideal IFN-free regimen, which should be a regimen with minimal pill burden and side-effects, limited drug–drug interactions, and the potency to cure almost all HCV-infected individuals, independently of previous treatment outcome or infection with distinct HCV genotypes? Currently, patients with a good responsiveness to IFN-α (e.g., previous relapsers, patients with good response *IL28B* genotype without significant liver fibrosis, or HCV genotype 2 and 3 infected patients) apparently have a good chance of cure with both triple therapy approaches as well as with selected all-oral regimens [4,33,50,58,66]. All-oral regimens for such "easy to cure" patients may include combinations of two DAAs (NS3-4A inhibitors, NS5A inhbitors, NIs or even NNIs), with, or – in selected regimens – even without ribavirin. While a high chance of cure in general is realistic in these patient populations with favorable baseline characteristics, further improvement is still relevant in terms of side-effects and convenience of available treatment options. However, in difficult-to-cure patients, such as patients with liver cirrhosis or HCV genotype 1a patients with previous null-response to pegIFN-α/ribavirin, most thus far investigated combinations of two DAAs ± ribavirin cannot prevent viral breakthrough in a significant proportion of patients. The addition of pegIFN-α and ribavirin as quadruple therapy approach has been proven highly effective to cure even null responders infected with HCV subtype 1a, but obviously this approach has a high risk of significant side effects and it is no option for patients not tolerating IFN-α [6,53]. Hence, it will be key to define DAA combination partners which could replace IFN-α for a successful all-oral therapy of these patients. Perhaps, optimized dual DAA regimens combining compounds with a very high antiviral activity (e.g., potent NS3-4A or NS5A inhibitors) together with a very high barrier to resistance (e.g., potent NIs like sofosbuvir or cyclophilin inhibitors) with or without ribavirin might still be successful even in difficult-to-cure HCV-infected patients. For example, the combination of daclatasvir plus sofosbuvir resulted in 100% cure in treatment-naïve HCV genotype 1 patients, independent of *IL28B* genotype or HCV subtype [60]. However, this combination has not been evaluated in previous null responders, and thus far no all-oral regimen achieving satisfying SVR rates in HCV genotype 1a null responders has been presented. Whether longer treatment durations, the combination of more DAAs (e. g., three DAAs plus ribavirin), application of even more potent DAAs, or perhaps the addition of alternative immune-modulatory agents can cure these patients is one of the most important questions for research in hepatology. Furthermore, it will be important to confirm efficacy and safety of already presented all-oral combinations in larger phase III clinical trials, as most studies evaluating IFN-free combinations thus far included only small numbers of highly selected patients (with the notable exception of Sound-C2, including 10% of cirrhotic patients of approximately 360 patients total [54]).

Several additional, more practical issues require attention for the further development of all-oral regimens. Importantly, most NS3-4A as well as NNIs have been developed exclusively for HCV genotype 1. Hence, the choice of DAA agents for treating HCV genotype 2–6 is currently restricted to other compounds, especially NS5A inhibitors, NIs, cyclophilin inhibitors, and some second-wave NS3-4A inhibitors [5]. Furthermore, possible drug–drug interactions within IFN-free regimens as well as with concomitant medications require attention [21]. Finally, a few cases of late relapses after all-oral as well as after triple-therapies are worrisome, possibly making a reassessment of the equitation of SVR at week 24 post treatment with cure from HCV infection necessary [58,67].

Conflict of interest

C.M.L.: Consultancy for Roche. S.Z.: Consultancy for Abbott, Achillion, Astrazeneca, BMS, Gilead, Idenix, Janssen, Merck, Novartis, Presidio, Roche, Santaris, and Vertex.

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