Interferon-Free Strategies without a Nucleoside/ Nucleotide Analogue

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Abstract

The identification of viral and host factors involved in hepatitis C virus (HCV) replication was a key prerequisite for the discovery and further exploration of antiviral drug targets. As of today, numerous direct-acting antiviral agents (DAAs), as well as host-targeting agents (HTAs), have been developed and entered clinical testing. The goal to omit pegylated interferon due to its unfavorable side-effect profile from novel HCV therapeutic approaches led to an expedited design and competitive conduct of DAA combination trials striving for easily applicable, all-oral HCV treatments. Approval of several interferon-free regimens is awaited in the near future (2014/2015). Results of different DAA combination trials (without nucleos(t)ide polymerase inhibitors) and trials involving HTAs are reviewed herein.

Keywords

- interferon-free HCV treatment
- direct-acting antiviral agents
- NS3/4A protease inhibitors
- NS5A inhibitors
- non-nucleoside polymerase inhibitors
- host-targeting agents

The establishment of a robust hepatitis C virus (HCV) cell culture system and subsequent detailed characterization of the HCV life cycle was a key step toward the identification of putative antiviral targets and respective antiviral drugs.[1,2] These include direct-acting antiviral agents (DAAs) such as NS3/4A protease inhibitors, NS5A inhibitors, nucleos(t)ide and non-nucleoside polymerase inhibitors, as well as host-targeting agents (HTAs) directed against cellular factors involved in viral entry or replication.[3] Trials investigating different drug classes with and without pegylated interferon (PegIFN) and/or ribavirin have rapidly evolved, yielding highly promising sustained virologic response (SVR) rates.[4,5]

In regard to the enormous public health impact of HCV infection with an estimated 184 million anti-HCV positive persons worldwide, the recent advances in HCV drug development, together with implementation of improved HCV screening programs, have the potential to substantially reduce the burden of HCV-associated advanced liver disease including hepatocellular carcinoma.[6–9]

The first-in-class NS3/4A inhibitors telaprevir and boceprevir were approved in 2011. Addition of these drugs to the PegIFN/ribavirin backbone has considerably improved response rates in HCV therapy naïve and experienced patients compared with PegIFN/ribavirin alone.[10–13]

The poor side-effect profile of these IFN-based therapies, however, limits the wide clinical applicability. Proof of the principle that viral clearance can be achieved with an IFN-free DAA combination has fast-tracked design and the conduct of clinical trials investigating IFN-free DAA combinations.[14] Different HCV genotypes and multiple subtypes result in a genotype and subtype-dependent efficacy of many available DAAs.[15,16] Furthermore, viral clearance is challenged by the presence and selection of resistance-associated variants (RAVs).[17,18]

Thus, essential prerequisites for DAAs in clinical development comprise a high antiviral efficacy, a broad and at best pan-genotypic activity, as well as a high barrier to resistance. In this respect, nucleos(t)ide polymerase inhibitors display very favorable characteristics.[19–21] In addition to a high antiviral efficacy, binding to the highly conserved active site of the RNA-dependent RNA-polymerase (RdRp) and incorporation as active triphosphates into the growing HCV RNA chain terminating its elongation, confers a pan-genotypic activity. Also, the low replication fitness of emergent

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HCV resistant variants clinically translates into a very favorable resistance profile of these drugs. In contrast, most non-nucleoside NS5B inhibitors have a lower antiviral efficacy.[19,22] Binding to different allosteric sites of the RdRp (thumb 1 and 2, palm 1 and 2) results in conformational changes. As these domains are highly variable across genotypes, use of currently available non-nucleoside NS5B inhibitors is restricted to treatment of HCV genotype-1 infection.[19] Also due to the drug classes low barrier to resistance, non-nucleoside polymerase inhibitors are so far only components in IFN-free multiclass DAA combination therapies.[19] Considerable progress was achieved with the ongoing development of NS3/4A and NS5A inhibitors. While the NS3/4A protease inhibitors telaprevir and boceprevir display a limited genotypic activity (mainly in genotype 1), simeprevir, faldaprevir, and ABT-450 have broader genotype coverage (genotypes 1, 2, 4, 5, 6). The low barrier to resistance of these NS3/4A protease inhibitors with largely overlapping resistance profiles remains a clinical challenge.[17,18] Simeprevir, faldaprevir, and ABT-450 have improved pharmacokinetic profiles allowing once-daily dosing.[23,24] Further advancement may be achieved with "second generation" protease inhibitors MK-5172 and ACH-2684.[25,26] These substances are thought to have a pan-genotypic activity and efficacy against variants that confer decreased susceptibility to first-generation protease inhibitors. In parallel, "second generation" NS5A inhibitors such as MK-8742 and ACH-3102 exhibit an improved antiviral efficacy, broader genotypic activity, and an improved barrier to resistance compared with first-generation NS5A inhibitors such as daclatasvir or ledipasvir.[27,28] In the following sections, we provide an overview of the results of DAA combination trials without a nucleoside/nucleotide analogue polymerase inhibitor (reviewed by J. Feld in this issue). DAAs in clinical development are displayed in ►**Table 1**. Selected clinical trials are summarized in ►**Table 2**.

Clinical Trials Investigating the Combination of a NS3/4A Protease Inhibitor and a Non-nucleoside Polymerase Inhibitor

Oral combination therapy with the NS3/4A protease inhibitor Faldaprevir and the nonnucleoside NS5B inhibitor deleobuvir was investigated in the SOUND-C2 trial.[29] In this open-label phase 2b study, 362 therapy naïve HCV genotype-1-infected patients (including cirrhotics) were randomly assigned to one of five different treatment groups receiving faldaprevir (120 mg once daily), deleobuvir (600 mg three times daily) and ribavirin for 16, 28, or 40 weeks (TID16W, TID28W, TID40W); faldaprevir (120 mg once daily) and deleobuvir (600 mg twice daily) and ribavirin for 28 weeks (BID28W), or faldaprevir (120 mg once daily) and deleobuvir (600 mg three times daily) without ribavirin for 28 weeks (TID28W-no ribavirin). SVR12 rates were highest in the BID28W group (69%) and ranged from 52 to 59% in the TID16–40W arms. Significantly lower response rates of 39%, however, were observed in the ribavirin-free TID28W-no ribavirin arm due to higher breakthrough and relapse rates. Analyses stratified by genotype clearly demonstrated a higher treatment efficacy in patients infected with HCV genotype 1b (SVR12 56–85%) compared with those infected with HCV genotype 1a (SVR12 11–47%), attributable to higher relapse rates in the latter group. Presence of IL28B CC genotype was a predictor of treatment response (SVR12 58–84% vs. 33–64% in IL28BCC vs. non-CC genotypes, respectively).

Another phase 2 trial (SOUND-C3) investigated faldaprevir (120 mg once daily), deleobuvir (600 mg twice daily), and ribavirin for 28 weeks in 20 therapy-naïve HCV genotype 1b patients and 12 patients with HCV genotype 1a expressing the IL28B CC genotype, including patients with liver cirrhosis.[30] SVR12 rates were 95% in patients with HCV genotype 1b infection, but only 17% in HCV genotype 1a-CC patients. Phase 3 programs, restricted to patients with HCV genotype 1b infection (HCVerso1, HCVerso2, HCVerso3) are currently ongoing, and results are not yet available (►**Table 2**).[31–33]

The combination of the ritonavir-boosted NS3/4A protease inhibitor ABT-450/r with one of two different NNIs (ABT-072 or ABT-333) was investigated in several phase 2 studies (PILOT, Co-PILOT, AVIATOR).[34–36] The PILOT-study was a single-arm trial investigating a 12-week combination therapy of ABT-450/r (150/100 mg once daily) and ABT-072 (400 mg once daily) and ribavirin in 11 therapy-naïve, non-cirrhotic HCV genotype 1 patients with the IL28B-CC genotype.[34] Of those 91% (10 of 11 patients) achieved an SVR24. One additional patient had a viral relapse 36 weeks after treatment discontinuation (SVR36 82%). The CO-PILOT study investigated two different doses of ABT450/r (250 mg/100 mg and 150 mg/100 mg) in combination with ABT-333 (400 mg twice daily) and ribavirin for 12 weeks in therapy-naïve HCV genotype 1-infected patients without cirrhosis (groups 1 and 2, $n = 19$ and $n = 14$ patients, respectively).[35] Group 3 ($n = 17$) included therapy-experienced patients (null or partial responders to prior PegIFN therapy) who were treated with ABT-450/r (150/100 mg once daily) and ABT-333 (400 mg twice daily) and ribavirin for 12 weeks. Response rates were 89%, 79%, and 47% for groups 1, 2, and 3, respectively.

The AVIATOR study enrolled noncirrhotic HCV genotype 1 therapy-naïve patients and patients with prior null response to a PegIFN-based therapy.[36] In 14 different treatment arms, different doses of ABT450/r (200/100 mg, 150/100 mg, or 100/100 mg once daily) were investigated in combination with ABT-333 (400 mg twice daily) or/and the NS5A inhibitor ABT-267 (25 mg once daily), with and without ribavirin. Therapy durations ranged from 8 to 24 weeks. Of 41 treatment-naïve patients who were randomized to treatment with ABT-450/r (150/100 mg once daily), ABT-333 and ribavirin for 12 weeks, 85.4% achieved an SVR12. Other study arms (combination of ABT450/r, ABT-267 and ribavirin, or triple DAA combinations) are reviewed below.

The ZENITH trial was designed to study combination therapy of the NS3/4A protease inhibitor telaprevir (1125 mg twice daily) and the non-nucleoside polymerase inhibitor lomibuvir (VX-222; 400 mg twice daily), with ribavirin in 23 HCV genotype 1a and 23 genotype 1b therapy-naïve patients without cirrhosis.[37] Patients with undetectable HCV RNA at

Table 1 Selected direct-acting antiviral agents and host-targeting agents (excluding nucleos(t)ide analogues)

Drug	Sponsor	Clinical development phase
Direct-acting antiviral agents		
NS3/4A protease inhibitors		
Linear		
Telaprevir (TVR)	Vertex/Janssen	Approved
Boceprevir (BOC)	Merck	Approved
ABT-450/r *	Abbvie	Phase 3
Asunaprevir (ASV, BMS-650032)	Bristol-Myers Squibb	Phase 3
Faldaprevir (BI 201335)	Boehringer-Ingelheim	Phase 3
Vaniprevir (MK-7009)	Merck	Phase 3
GS-9451	Gilead	Phase 2
Sovaprevir (ACH-1625)	Achillion	on hold
Narlaprevir (SCH 900518)	Merck	Phase 2
Macrocyclic		
Danoprevir (DNV/r, RG-7227) *	Roche	Phase 3
Simeprevir (TMC435)	Janssen	Approved
GS-9256	Gilead	Phase 2
MK-5172	Merck	Phase 2
ACH-2684	Achillion	Phase 1
BZF-961	Novartis	Phase 1
NS5A Inhibitors		
Daclatasvir (DCV, BMS-790052)	Bristol-Myers-Squibb	Phase 3
ABT-267	Abbvie	Phase 3
Ledipasvir (LDV, GS-5885)	Gilead	Phase 3
GS-5816	Gilead	Phase 2
PPI-668	Presidio	Phase 2
GSK2336805	GlaxoSmithKline	Phase 2
MK-8742	Merck	Phase 2
ACH-3102	Achillion	Phase 2
EDP239	Novartis	Phase 1
PPI-461	Presidio	Phase 1
IDX719	Idenix Pharmaceuticals	Phase 1
Non-nucleoside polymerase inhibitors		
Palm I		
ABT-333	Abbott	Phase 3
ABT-072	Abbott	Phase 2
Setrobuvir (ANA-598, RO5466731)	Roche	Phase 2
Palm II		
Tegobuvir (GS-9190)	Gilead	Discontinued
Thumb I		
Deleobuvir (BI 207127)	Boehringer-Ingelheim	Phase 3
BMS-791325	Bristol-Myers-Squibb	Phase 2
TMC647055	Medivir	Phase 2
Thumb II		

(*Continued*)

Table 1 (*Continued*)

Drug	Sponsor	Clinical development phase
Lomibuvir (VX-222)	Vertex	Phase 2
GS-9669	Gilead	Phase 2
Host-targeting agents (HTAs)		
Alisporivir	Novartis	Phase 2
Miravirsen	Santaris	Phase 2

Abbreviations: * /r, Ritonavir-boosted.

week 4 and 8 (26% and 22% of the genotype 1a and genotype 1b patients, respectively) were eligible to discontinue treatment at week 12. Of those, 73% genotype 1a and 71% genotype 1b patients achieved an SVR12. The ribavirin free-treatment arms were discontinued due to high breakthrough rates meeting predefined stopping criteria.

The phase 2 ANNAPURNA study tested the combination of the ritonavir-boosted NS3/4A protease inhibitor danoprevir, the non-nucleoside polymerase inhibitor setrobuvir, and mericitabine, a nucleoside analog polymerase inhibitor and ribavirin for 12 to 24 weeks in noncirrhotic treatment-naïve HCV genotype-1-infected patients.[38] The combination of DNV/r (100/100 mg twice daily), setrobuvir (800 mg twice daily on day 1, followed by 400 mg twice daily thereafter), and ribavirin was investigated in genotype 1a patients for 24 weeks (Arm C), and in genotype 1b patients for 12 weeks (Arm E). Treatment Arm C met a predefined futility rule due to a high viral breakthrough rate. SVR rates for Arm E were reported with 77.7% at The American Association for the Study of Liver Diseases (AASLD) 2013.[38]

Another study, combining the NS3/4A protease inhibitor simeprevir and TMC647055, a non-nucleoside polymerase inhibitor and low-dose ritonavir with and without ribavirin for 12 weeks is currently planned (►**Table 2**).[39]

Clinical Trials Investigating the Combination of a NS3/4A Protease Inhibitor and a NS5A Inhibitor ± Ribavirin

Proof of concept that eradication of HCV is possible by the combination of a NS3/4A protease inhibitor and a NS5A inhibitor was first demonstrated in a small exploratory trial.[14] In this study, 11 HCV genotype 1 null responders to prior PegIFN/ ribavirin therapy were treated with the NS3/4A protease inhibitor asunaprevir (ASV, 600 mg twice daily) and the first-in-class NS5A inhibitor daclatasvir (DCV, 60 mg once daily) for 24 weeks. Overall, 36% of the patients achieved an SVR. Virologic response rates differed by HCV subtype, as viral clearance was achieved in two of two HCV genotype 1b-infected patients, but in only two of nine (22, 2%) HCV genotype-1a-infected patients due to high viral breakthrough rates. Therapeutic efficacy of this dual DAA regimen administered for 24 weeks was further investigated in several phase 2 trials conducted in Japan and in the United States.[40–42]

Two Japanese studies enrolled HCV genotype 1b patients without cirrhosis who were intolerant/ineligible and/or null-responder patients.[40,41] Response rates in prior null responders, as reported in one of the trials, were 100%; in the other trial, response rates of 63.6% and 90.5% were reported in PegIFN intolerant/ineligible ($n = 22$) and null-responder ($n = 10$) patients, respectively.[40,41]

Of note, in these Japanese phase 2 trials asunaprevir dose was adjusted from 600 mg twice daily to 200 mg twice daily due to observed hepatotoxicity at higher doses.[43] The U.S. study investigated different dosing regimens of asunaprevir (200 mg twice daily vs. 200 mg once daily) in HCV genotype-1b null-responders without cirrhosis: SVR rates ranged from 65 to 78%.[43]

At AASLD 2013, SVR24 results of a Japanese phase 3 trial were reported to be 87.4% in IFN-ineligible/intolerant patients ($n = 135$) and 80.5% in prior null-responders ($n = 87$) who received asunaprevir and daclatasvir (100 mg twice daily and 60 mg once daily) for 24 weeks.[44] Importantly, baseline factors such as compensated liver cirrhosis did not affect response rates. Another phase 3 trial ("Hallmark-Dual") currently investigates the same regimen in therapy-naïve patients, prior null-/partial-responders and patients with PegIFN ineligibility or intolerance; results were not yet presented.[45]

Efficacy of daclatasvir in combination with the NS3/4A protease inhibitor simeprevir is currently studied in a pharmaceutical cross-collaboration.[46] In this open-label phase 2 study, 140 HCV genotype 1b-infected, therapy-naïve patients or patients with prior null response to PegIFN and ribavirin therapy were randomized to receive DCV (30 mg once daily) and simeprevir (150 mg once daily) either with or without ribavirin for 12 weeks. Patients with compensated cirrhosis (up to 35%) were included. Patients were re-randomized at week 12 to stop therapy or continue for another 12 weeks. A small pilot cohort of HCV genotype 1a-infected patients was included in this study, who received simeprevir and daclatasvir in combination with ribavirin for 24 weeks. Results of this trial are not yet available.

The AVIATOR study, as outlined above, also investigated the efficacy of ABT-450/r, the NS5A inhibitor ABT-267, and ribavirin.[36] Seventy-nine therapy-naïve HCV genotype 1 patients and 45 prior null responders were treated with ABT-450/r (100/100 mg and 200/100 mg once daily for naïve and 200/100 mg once daily for prior null responders) and ribavirin for 12 weeks. SVR24 rates were as high as 89.9% and 88.9%, respectively. Results of the PEARL-1 study that investigated the efficacy of a ribavirin sparing combination of ABT-450/r (150/100 mg once daily) and ABT-267 (25 mg once daily) for 12 weeks were reported at AASLD 2013.[47] SVR12 rates in 42

Table 2 Overview of selected DAA combination therapies without a nucleos(t)ide polymerase inhibitor

Sponsor	DAA combination / RBV	Phase	Patient population	Therapy duration (weeks)	SVR (%)
NS3/4A protease inhibitor (PI) + non-nucleoside polymerase inhibitor ± RBV					
Boehringer-Ingelheim	Faldaprevir + Deleobuvir ± RBV (SOUND-C2)	2	GT1, naïve, ± cirrhosis	16–40	GT1a: 11–47 GT1b: 56–85
	Faldaprevir + Deleobuvir + RBV (SOUND-C3)	2	GT1a (*IL28B* CC), GT1b, naïve ± cirrhosis	16	GT1a (*IL28B* CC): 16.6 GT1b: 95
	Faldaprevir + Deleobuvir + RBV (HCVerso1)	3	GT1b, naïve ± cirrhosis	16–24	Ongoing
	Faldaprevir + Deleobuvir + RBV (HCVerso2)	3	GT1b, naïve, ineligible ± cirrhosis	16–24	Ongoing
	Faldaprevir + Deleobuvir + RBV (HCVerso3)	3	GT1b, naïve, PegIFN intolerant, prior relapse, partial response + cirrhosis (CHILD A/B)	24	Ongoing
Abbvie	ABT-450/r + ABT-072 +RBV (PILOT)	2	HCV GT1, naïve, no cirrhosis	12	91
	ABT-450/r + ABT-333 +RBV (CO-PILOT)	2	HCV GT1, naïve, prior null or partial response, no cirrhosis	12	Naïve: 79–89 Experienced: 47
	ABT-450/r + ABT-333 +RBV (AVIATOR)	2	HCV GT1, naïve, no cirrhosis	12	85.4
Vertex	Telaprevir + VX222 + RBV (ZENITH)	2	HCV GT1, naïve, no cirrhosis	12	GT1a: 73 GT1b: 70
Roche	Danoprevir/r + Setrobuvir + RBV (ANNAPURNA)	2	HCV GT1, naïve, no cirrhosis	12–24	GT1a: futility rule GT1b: 77.4 (SVR4)
Janssen	Simeprevir + TMC647055, low-dose ritonavir	2	HCV GT1, naïve, no cirrhosis	12	Planned
NS3/4A protease inhibitor + NS5A inhibitor ± RBV					
Bristol-Myers Squibb	Asunaprevir + Daclatasvir	2	GT1, prior null responder, no cirrhosis	24	GT1a: 22 GT1b: 100
	Asunaprevir + Daclatasvir (Japan)	2	GT1b, prior null responder, no cirrhosis	24	100
	Asunaprevir + Daclatasvir (Japan)	2	GT1b, prior null responder; PegIFN intolerant/ineligible, no cirrhosis	24	Null responder: 90.5 Ineligible/intolerant: 63.6
	Asunaprevir + Daclatasvir (US)	2	GT1b, null responder, no cirrhosis	24	65–78
	Asunaprevir + Daclatasvir (Japan)	3	GT1b, PegIFN intolerant/ineligible, prior null responder ± cirrhosis	24	Ineligible/intolerant: 87.4 Null responder 80.5
	Asunaprevir + Daclatasvir (Hallmark-Dual)	3	GT1b, naïve, PegIFN intolerant/ineligible, prior null-/partial responder, ± cirrhosis	24	Ongoing
Janssen and Bristol-Myers Squibb	TMC435 + Daclatasvir ± RBV	2	GT1, naïve, prior null responder ± cirrhosis	12–24	Ongoing
Abbvie	ABT-450/r + ABT-267 + RBV (AVIATOR)	2	GT1, naïve, prior null responder, no cirrhosis	12	Naïve: 89.9 Null responder 88.9
	ABT-450/r + ABT-267 ± RBV (PEARL-1)	2	HCV GT1b, naïve, null responder, no cirrhosis	12	Naïve: 95.2 Null responders: 90
	ABT-450/r + ABT-267 + RBV	3	GT2, naïve, experienced ± cirrhosis	12–16	Planned
	ABT-450/r + ABT-267 + RBV	3	GT3, naïve, experienced ± cirrhosis	16–24	Planned

(*Continued*)

Table 2 (*Continued*)

Sponsor	DAA combination / RBV	Phase	Patient population	Therapy duration (weeks)	SVR (%)
Merck	MK-5172 + MK-8742 ± RBV (C-WORTHY)	2	GT1, naïve, no cirrhosis	12	89–100
NS3/4A Protease inhibitor + NS5A inhibitor + non-nucleoside polymerase inhibitor ± RBV					
Boehringer-Ingelheim	Faldaprevir + PPI-668 + Deleobuvir ± RBV	2	GT1a, naïve, no cirrhosis	12	100% SVR4
Bristol-Myers Squibb	Asunaprevir + Daclatasvir + BMS-791325	2	GT1, naïve, no cirrhosis	12–24	92
	Asunaprevir + Daclatasvir + BMS-791325	2	GT1, naïve, + cirrhosis	12	86.4 (cirrhosis) – 92.3
Abbvie	ABT-450/r + ABT-267 + ABT-333 ± RBV (AVIATOR)	2	GT1, naïve, null responder, no cirrhosis	8–24	Naïve: 87–97.5% Null responder 93.3%
	ABT-450/r + ABT-267 + ABT-333 + RBV (SAPPHIRE-I)	3	GT1, naïve, no cirrhosis	12	Ongoing
	ABT-450/r + ABT-267 + ABT-333 + RBV (SAPPHIRE-II)	3	GT1, experienced (null / partial responder, relapse), no cirrhosis	12	Ongoing
	ABT-450/r + ABT-267 + ABT-333 + RBV (TURQUOISE)	3	GT1 naïve, experienced (null / partial responder, relapse) + cirrhosis	12–24	Ongoing
	ABT-450/r + ABT-267 + ABT-333 ± RBV (PEARL-II)	3	GT1b, experienced, no cirrhosis	12	Ongoing
	ABT-450/r + ABT-267 + ABT-333 ± RBV (PEARL-III)	3	GT1b, naïve, no cirrhosis	12	Ongoing
	ABT-450/r + ABT-267 + ABT-333 ± RBV (PEARL-IV)	3	GT1a, naïve, no cirrhosis	12	Ongoing

Abbreviations: DAA, direct-acting antiviral agent; HCV, hepatitis C virus; PegIFN, pegylated interferon; SVR, sustained virological response.

treatment-naïve and 40 prior null responders were 95.2% and 90.0%, respectively.

At AASLD 2013, promising results were also presented from the C-WORTHY trial.[48] This phase 2 study investigated safety and efficacy of a 12-week combination therapy with MK-5172, a second generation NS3/4A protease inhibitor, and MK-8742, a potent second-generation NS5A inhibitor, with or without ribavirin in 65 treatment-naive, noncirrhotic patients infected with HCV genotype 1. Patients with HCV genotype 1b infection were randomized 1:1:2 into one of three treatment arms where they received MK5172 100 mg once daily and MK8742 20 mg or 50 mg once daily plus ribavirin (arms 1 and 2) or the ribavirin-free dual DAA combination of MK5172 (100 mg once daily) and MK8742 (50 mg once daily, arm 3). Patients infected with HCV genotype 1a were only allocated to ribavirin containing arms. SVR12 rates were 96%, 89%, and 100% for arms 1, 2, and 3, respectively. Importantly, response rates in arm 1 and 2 were found to be equally effective in HCV genotype 1a and genotype 1b patients. Also, addition of ribavirin did not provide an additional benefit for patients infected with HCV genotype 1b.

A double-blind, placebo-controlled phase 2 trial evaluated the safety, tolerability, and efficacy of 12 weeks of sovaprevir, another second-generation protease inhibitor, ACH-3102, an NS5A inhibitor and ribavirin in up to 50 treatment-naïve patients with HCV genotype 1 infection. Sovaprevir, however, was placed on partial clinical hold by the FDA due to liver enzyme elevations in a phase 1 drug–drug interaction study with ritonavir-boosted atazanavir. Treatment of patients in the above-mentioned study was continued, results have not yet been disclosed.[49]

Clinical Trials Investigating Triple DAA Combination Therapies

The combination of three DAAs, including an NS3/4A protease inhibitor, an NS5A inhibitor, and a non-nucleoside polymerase inhibitor, with or without ribavirin, has been investigated in several studies. Interim results of a phase 2 trial exploring whether the addition of the NS5A inhibitor PPI-668 to faldaprevir and deleobuvir may improve response rates in HCV genotype 1a patients were presented recently.[50] Twenty-four HCV genotype 1a-infected therapy naïve patients without cirrhosis were randomized to one of two 12-week treatment cohorts where they received faldaprevir (120 mg once daily), PPI-668 (200 mg once daily), and two different doses of deleobuvir (600 mg twice daily or 400 mg twice daily; cohort 1 and 2, respectively). A subset of the patients ($n = 13$) was allocated to a 12-week, ribavirin-free triple DAA treatment arm (cohort 3). At the time of the AASLD 2013 presentation one patient harboring the NS5A variant Q30L-Y93H at baseline had experienced viral breakthrough after week 5, and 13 patients had achieved SVR4.

Another phase 2, open-label study examined the ribavirin-free combination of daclatasvir (60 mg once daily), asunaprevir (200 mg twice daily) and the non-nucleoside NS5B inhibitor BMS-791325 (75 or 150 mg twice daily) for 12 or 24 weeks in 66 treatment-naïve HCV genotype-1-infected patients without cirrhosis.[51] Based on a modified intent-to-treat analysis, 92% of the patients achieved an SVR12. Results of an expansion cohort evaluating this triple DAA regimen in a larger number ($n = 166$) of therapy-naïve patients including cirrhotic patients were presented at AASLD 2013.[52] SVR4 rates were similar in the BMS-791325, 150 mg, and 75 mg groups (91.7% and 92.3%, respectively); 86.4% (13 of 15 patients) of the cirrhotic patients achieved an SVR4. Trials assessing this combination in mainly HCV genotype-4-infected patients and in prior null responders are currently planned.

In the AVIATOR study, triple DAA therapy with ABT450/r, ABT-267, and ABT-333 in combination with ribavirin for 12 weeks yielded high SVR rates of 97.5% and 93.3% in HCV genotype 1-infected, therapy-naïve patients and null responders to prior PegIFN/ribavirin therapy, respectively.[36] Sustained virologic response rates were lower in the 8-week arm (87.5%) as well as in a ribavirin-sparing therapy arm (87.3%). Ribavirin dose reductions mainly due to hemolytic anemia did not impact SVR rates.[53]

Ongoing phase 3 studies investigate virologic response rates of these triple DAA regimens in combination with ribavirin in HCV-genotype-1 infected, noncirrhotic, therapy naïve and experienced patients (SAPPHIRE-1 and SAPPHIRE-II) or in patients with cirrhosis (TURQUOISE-II) (►**Table 2**).[54–56] Preliminary results from the SAPPHIRE-1 and SAPPHIRE-II study were released recently. Overall SVR rates were 96% in both trials, and final results are awaited.[57]

Also, treatment efficacy of ABT450/r, ABT-267, and ABT-333 is currently studied with and without ribavirin in HCV genotype-1b-infected therapy naïve and experienced patients (e.g., PEARL-II, PEARL-III) and in patients with HCV genotype 1a infection (PEARL-IV).[58–60]

DAA Monotherapy

Data on prolonged monotherapy with an NS3/4A protease inhibitor are only available from a small Japanese study investigating telaprevir monotherapy for 24 weeks in patients infected with HCV genotype 1b.[61] Response rates were very unfavorable due to the rapid selection of RAVs. Until recently, the concept of NS3/4A monotherapy was not further pursued. At AASLD 2013, however, highly interesting data of the C-SPIRIT study were presented.[62] This study investigated the oral combination of the second-generation NS3/4A polymerase inhibitor MK-5172 (100 mg once daily) and ribavirin for 12 or 24 weeks (arm 1 and 2, respectively) in 25 noncirrhotic HCV genotype 1 patients with an IL28B CC genotype. Patients in arm 1 who achieved an RVR (HCV RNA undetectable at week 4) were eligible to stop treatment at week 12. Four out of five HCV genotype 1b and one out of three genotype 1a patients achieved an SVR12. At the time of presentation, SVR12 rates were not yet available for the 24-week treatment arm, but so far, 12/15 (80%) of the patients achieved an SVR4. Results of this study are certainly stimulating, as they provide first evidence that second-generation protease inhibitors with a high barrier to resistance may be successful in combination with only ribavirin in selected patients.

Host-Targeting Agents

The establishment of robust cell culture systems for HCV propagation facilitated the identification of investigational HTAs. In contrast to DAAs, which exert their functions by targeting viral proteins, HTAs unfold their actions by blocking host cell structures involved in the biological life cycle of HCV. These different mechanisms explain why HTAs are less susceptible to HCV genotypic differences and emergence of viral resistance.

HTAs inhibit for example viral entry factors, kinases, micro-RNAs, or enzymes involved in viral replication (reviewed in[63,64]). Monoclonal antibodies directed against viral entry factors (e.g., CD81-mAbs, anti-SR-BI mAbs, ITX5061), or EGFR kinase (erlotinib) and the cholesterol absorption NPC1L1 inhibitor ezetimibe have been shown to protect or delay HCV infection in mice.[65–70] As of today, development of these compounds (except ITX5061) is yet in preclinical stages. In a recent phase 1b study ITX5061, however, did not meet protocol-defined criteria for viral suppression.[71]

Eucaryotic micro-RNAs are well conserved noncoding RNA molecules (~20–22 nucleotides in length) that regulate transcriptional and posttranscriptional gene expression by targeting mRNAs.[71] miR-122 is a liver-specific micro RNA with a pivotal role in HCV RNA replication.[72] miR-122 target binding sites in the HCV 5′-UTR are highly conserved across all HCV genotypes. The miR-122–HCV interaction is complex and amongst others, protects the cytosolic RNA from exonuclease digestion and activation of innate RNA-triggered immune responses. It is required for efficient HCV RNA replication.

A recent phase 2 study investigated the efficacy of miravirsen, a miR-122 antagonist in 36 therapy-naïve HCV genotype 1-infected patients.[73] Study participants were randomized to receive five weekly doses of miravirsen (3 mg, 5 mg, or 7 mg per kilogram of body weight) or placebo subcutaneously. Administration of miravirsen resulted in a dose-dependent reduction in HCV RNA of up to 3 $\log_{10}$ IU per milliliter in patients randomized to the highest dose group.

Alisporivir (ALV, DEB025) is a first-in class oral HTA that inhibits cyclophilin A, a cellular enzyme with isomerase and chaperone activity. Cyclophilin A has been shown to play an important role in HCV replication and assembly through interaction with HCV NS5A and NS5B proteins. Although alisporivir is a structural analog of cyclosporine A, the alisporivir-cyclophilin complex does not inhibit calcineurin, and therefore lacks immunosuppressive activity.[74–76] Alisporivir is active against all HCV genotypes and has a high barrier to resistance.[77] A temporary U. S. Food and Drug Administration hold was placed 2012 on the alisporivir development program due to safety concerns arising in alisporivir/PegIFN combination trials. Safety issues included hematologic toxicities, hypertriglyceridemia, and six severe cases of pancreatitis, including one death. Extensive safety analyses have moved the lately resumed alisporivir development program onto the IFN-free track. Only recently, a randomized phase 2 trial was initiated to investigate response-guided therapy with alisporivir (200 mg, 300 mg, 400 mg) plus ribavirin for 12–24 weeks in therapy naïve, HCV genotype 2/3-infected patients.[78] This study was motivated by results of the phase 2 VITAL-1 study that demonstrated SVR12 rates of $>$ 80% in patients with RVR receiving alisporivir in combination with ribavirin for 24 weeks.[79] A recent in vitro study demonstrated synergistic antiviral activity of the combination of alisporivir and NS5A inhibitors.[80] These findings may stimulate further clinical investigation of IFN and possibly ribavirin-free alisporivir–NS5A inhibitor combination therapies. Other cyclophilin A inhibitors such as SCY-635 and EDP-546 are in early clinical development.[81,82]

Summary and Perspectives

HCV drug development is currently on a competitive speedway toward the ultimate goal of a safe, well-tolerable, IFN-free, all-oral, pan-genotypic HCV therapy. Approval of next-generation protease inhibitors such as simeprevir, faldaprevir, and asunaprevir is expected in 2014. The very recent approval of sofosbuvir, a nucleotide polymerase inhibitor that, in combination with ribavirin, shows excellent efficacy in HCV genotype 2 and 3 infections, and together with PegIFN ~ 90% SVR rates for patients infected with HCV genotype 1, has again changed the current HCV treatment landscape the current HCV treatment landscape. Other PegIFN-free DAA combinations that include for example, faldaprevir and deleobuvir, asunaprevir and daclatasvir, simeprevir and daclatasvir, as well as ABT-450, ABT-267, and ABT-333 will likely become available late 2014/early 2015. Without the backbone of potent nucleotide polymerase inhibitors, combinations of two or more DAAs will be required to maximize efficacy and minimize the emergence of RAVs, particularly in difficult to treat genotypes such as HCV genotype 1a. Second-generation NS3/4A protease inhibitors and potentially NS5A inhibitors may also exhibit high barriers to resistance.

As to ribavirin, its relevance varies among different DAA combinations tested. Available data suggest that it particularly benefits treatment of more difficult-to-cure subtypes such as HCV genotype 1a. It should be noted, that efficacy of several DAA combinations are either not yet examined in patients with liver cirrhosis, or results of these studies are still pending. Generally, future efforts in HCV drug development should essentially focus on the safety and efficacy in patients with more advanced liver disease and other special populations such as patients with end-stage renal failure, transplanted patients, and HIV/HCV coinfected patients. The future role of HTAs still needs to be defined.

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