The results from clinical trials testing new direct-acting antivirals (DAAs) for chronic hepatitis C were the major focus of interest at the 2012 annual meeting of the European Association for the Study of the Liver. Besides triple combinations, in which any one of the new DAAs is given along with peginterferon-α/ribavirin, clinical trials exploring interferon-free oral regimens combining several DAAs attracted major attention. The good tolerance, broad hepatitis C virus (HCV) genotype activity, and high resistance barrier of sofosbuvir make this nucleotide analogue one of the most promising DAAs. Among HCV protease inhibitors, the safety, potency, and convenient dosing of simeprevir, asunaprevir, faldaprevir, and ABT-450/r were particularly highlighted. Among NS5A inhibitors, the good performance of daclatasvir encourages further clinical development. Finally, intriguing results were released about the role of interleukin 28B (IL-28B) polymorphisms using interferon-free regimens, indirectly supporting the role of innate immunity for clearing HCV definitively.

**Keywords.** hepatitis C; directly acting antivirals; DAA; HIV; drug resistance.

The 47th Annual Meeting of the European Association for the Study of the Liver (EASL) in April 2012 brought together more than 9000 participants in Barcelona, Spain. This year’s event was the largest to date, mainly reflecting the huge interest in hepatitis C virus (HCV) treatment spurred by the arrival of the first direct-acting antivirals (DAAs).

The approval of telaprevir and boceprevir has heralded a new era that somewhat resembles what happened in 1996 in the AIDS field, following the approval of the first human immunodeficiency virus (HIV) protease inhibitors and triple combination therapy. Advances in therapeutics have concurred with the introduction of new diagnostic tools for hepatitis C. Two major breakthroughs merit particular recognition. Liver biopsies are no longer required for assessing the severity of hepatic damage caused by HCV. Noninvasive tools, including serum fibrosis indexes and, in particular, elastometry, now allow rapid, cheap, and accurate assessment of the extent of liver fibrosis in a given patient. In contrast with liver biopsies, these procedures can be repeated periodically. Elastometry is widely used in Europe, but it has still limited implementation in the United States. Prioritization of treatment in subjects with advanced liver fibrosis is justified in these first years of use of DAAs, given the complexity of dosing, frequent side effects, and high cost. However, as therapies for hepatitis C become simpler, it is likely that most, if not all, individuals with HCV will be considered as candidates for treatment, regardless of liver fibrosis stages [1]. Another landmark discovery for hepatitis C comes from genetics. Polymorphisms at the interleukin 28B (IL-28B) gene largely influence treatment responses, highlighting the role of the innate immunity for HCV clearance [2].
HCV PROTEASE INHIBITORS

No randomized clinical trials have compared so far the first marketed DAAs. However, the large number of patients already treated with telaprevir (Janssen) and boceprevir (Merck) allows drawing some comparisons. The CUPIC (Compassionate Use of Protease Inhibitors in Cirrhotics) study examined the performance of telaprevir and boceprevir in the French early access program in patients with compensated cirrhosis [3]. An interim analysis at week 16 of treatment was performed on 310 patients, 176 on telaprevir and 134 on boceprevir. Undetectable viremia was recognized in 71% and 61% of patients, respectively. Discontinuations due to serious adverse events occurred in 12% and 6%, respectively. The incidence of grade 3/4 hematological toxicities was fairly comparable: anemia (53% vs 66%), neutropenia (12% vs 11%), and thrombocytopenia (22% vs 8%). Grade 3 rashes occurred in 7% of patients on telaprevir but in none on boceprevir. Given that all patients had finished the planned 12 weeks on telaprevir but still had to complete treatment with boceprevir, these preliminary results should be interpreted with caution, as more patients on boceprevir would require stopping the medication prematurely owing to adverse events. Overall, CUPIC highlights the high burden of toxicities associated with current triple therapy in patients with cirrhosis, a population underrepresented in clinical trials but at first row for treatment at HCV clinics.

The PROVIDE study analyzed the efficacy of boceprevir in interferon (IFN) α–experienced patients [4]. Boceprevir administration was preceded by a lead-in phase of 4 weeks. Of particular interest was the information from prior null responders, given that this subset of patients had been excluded in registration trials. Sustained virologic response (SVR) occurred in 55% and 36% of prior null responders who achieved more or less than 1 log decline in serum HCV RNA during the lead-in phase. The 56% SVR rate in relapsers was low and unexpected, most likely explained by the small size of the study population.

Updated information on boceprevir therapy along with pegylated IFN-α/ribavirin (pegIFN-α/RBV) in 98 HIV/HCV–coinfected patients was presented [5]. The rate of SVR 12 weeks after completion of treatment (SVR12) was 63%, whereas it was 27% in pegIFN-α/RBV controls. Most patients in this trial received HIV protease inhibitors, for which a contraindication has recently been released owing to significant pharmacokinetic interactions, resulting in reduced exposure to both boceprevir and HIV protease inhibitors [6]. However, HIV rebounds in this trial did not differ comparing treatment arms. The frequency and modality of side effects in the coinfected population resembled what already has been reported in HCV-monoinfected patients (ie, anemia, neutropenia, dysgeusia, vomiting, diarrhea).

Danoprevir (Roche) is a potent macrocyclic HCV protease inhibitor active against genotypes 1, 4, and 6. Coadministration with ritonavir 100 mg daily improves danoprevir pharmacokinetics and toxicity profile. The drug is mostly metabolized by CYP450-A3 [7]. In the DAUPHINE trial, a phase 2b study, the 2 arms using danoprevir/ritonavir 100 or 200 mg daily for 24 weeks along with pegIFN-α/RBV provided SVR12 rates of 77% and 86%, respectively [8]. Differences were noticed between HCV subtype 1a (77% and 90%), subtype 1b (96% and 97%), and genotype 4 (100% for both doses). Diarrhea was the only adverse event directly attributed to danoprevir.

Simeprevir (Janssen, formerly TMC-435) is currently completing phase 3 trials. The ASPIRE trial [9] examined 2 doses of the drug (100 or 150 mg daily) along with pegIFN-α/RBV for 48 weeks in IFN-α–experienced patients. Overall SVR rates were 51% in null responders, 75% in partial responders, and 85% in relapers. Even with this potent drug, patients infected with HCV subtype 1a and/or cirrhosis responded less well than the rest, although this effect was not recognizable in relapers. Jaundice was the only remarkable side effect of simeprevir.

ABT-450 (Abbott) is administered with 100 mg of ritonavir, allowing once-daily dosing. In a phase 2a trial, SVR was attained by 14 of 16 patients who received triple therapy with ABT-450 (100 or 200 mg) plus ritonavir, followed by pegIFN-α/ RBV until completion of 48 weeks [10]. The 2 failures corresponded to 1 relapsing subject and another lost to follow-up after ending treatment with undetectable serum HCV RNA. Of note, IL-28B variants did not influence treatment outcomes and tolerance was similar in ABT-450 and control arms. The dose of ABT-450/ritonavir 150/100 mg daily has been selected for further clinical development.

NUCLEOS(T)IDE ANALOGUES

Agents within this class act as chain terminators blocking nascent HCV RNA elongation by the HCV polymerase. In contrast with other DAAs, these molecules exhibit in vivo a unique high barrier to resistance (Table 1). Mericitabine (Roche) is a cytidine analogue that blocks the HCV polymerase by competitive inhibition. The combination of mericitabine and danoprevir without IFN-α was the first to prove that all-oral regimens could completely suppress HCV replication in vivo [11]. However, in another IFN-α–free study, treatment for 12–24 weeks with mericitabine plus danoprevir/ritonavir with or without RBV demonstrated suboptimal efficacy in IFN-α–naive HCV-1 patients. The 12-week treatment duration arm was prematurely stopped due to high relapse rate (71% in HCV-1b and 41% in HCV-1a) [12].

Sofosbuvir (formerly GS-7977, Gilead) is a uridine analogue and currently one of the most attractive DAAs. It displays potent antiviral activity (4–5 log drop in serum HCV...
RNA on average as monotherapy). Several phase 2 trials have tested its activity and safety along with pegIFN-α (studies PROTON and ATOMIC) or without pegIFN-α (studies ELECTRON and QUANTUM). Triple therapy including pegIFN-α/RBV and sofosbuvir cures ≥90% of patients treated for 12 or 24 weeks regardless of HCV genotype [13, 14]. The enthusiasm that followed the release of early results using sofosbuvir plus RBV for 12 weeks in patients with HCV genotypes 2/3 (100% SVR) [15] has been tempered when results for HCV genotype 1 have been reported, either for IFN-α–naïve patients (average SVR 76%) [16] or prior null responders (only 11% SVR) [17]. Interestingly, failures result from relapses, as all patients treated reach undetectability. Moreover, relapses mainly occur in patients with unfavorable IL-28B alleles. This finding supports that definitive HCV clearance requires the contribution of immunity on top of complete viral suppression.

There are several features that make sofosbuvir particularly attractive as a component of new combination therapies for hepatitis C. First is its good safety profile, with lack of reports of any serious adverse event in the first 450 patients treated for at least 12 weeks [18]. A second advantage of sofosbuvir is the lack of selection of drug resistance mutants in vivo in patients who fail treatment. Last, the drug is prescribed as a convenient once-daily single pill with no evidence so far for significant drug interactions. Thus, patients with severe hepatic insufficiency [19] or receiving other oral antivirals [20] might be treated with sofosbuvir.

**NONNUCLEOSIDE HCV POLYMERASE INHIBITORS**

The performance of this family of compounds has mostly been examined as part of IFN-α–free regimens. Results from the most promising trials with these agents were presented at EASL. The SOUND-C2 trial examined the combination of BI-7127 (Boehringer) with the protease inhibitor faldaprevir for 16, 28, or 40 weeks, with and without RBV [21]. SVR12 ranged from 49% to 69% in arms that received RBV, but dropped to 39% in patients only receiving dual oral therapy. No severe hematological adverse events were recorded, with rash, jaundice, and gastrointestinal complaints being the most common toxicities. Of note, patients with HCV-1b responded much better than those with HCV-1a. In 37 patients with cirrhosis, triple therapy gave an SVR12 of 60% in HCV-1a and 83% in HCV-1b [22].

The combination of telaprevir with VX-222 (Vertex) was examined in the phase 2a ZENITH trial [23]. Three arms were tested, including for the first 12 weeks dual therapy with both DAAs, triple therapy adding RBV, and quadruple therapy also including pegIFN-α. All patients completed 48 weeks with pegIFN-α/RBV. Overall, 17% and 31% of patients experienced early viral breakthrough in the dual arms, forcing premature interruption of these groups. Most failures occurred in patients infected with HCV subtype 1a carrying IL-28B non-CC variants. Resistance mutations at both the protease (R155T/K, A156T) and polymerase (mainly R422K) were selected in all failures.

**ABT-072** and **ABT-333** (Abbott) are 2 potent nonnucleoside analogues that inhibit the HCV polymerase. In a phase 2b trial, testing these drugs separately as part of triple therapy with pegIFN-α/RBV for 12 weeks, with completion of 48 weeks of therapy with pegIFN-α/RBV, SVR was 85% with ABT-072 and 63% with ABT-333 [24]. No severe adverse effects nor treatment discontinuations due to toxicities were recorded, although only 39 patients were recruited in the study.

Both ABT-072 and ABT-333 have also been tested along with ABT-450/ritonavir as part of IFN-α–free combinations. In IFN-α–naïve, noncirrhotic, IL-28B CC patients, the SVR was 82% with ABT-072, ABT-450/ritonavir, and RBV; all failed treatment owing to relapses [25]. Better results were achieved with ABT-333 given along with ABT-450/ritonavir and RBV (SVR 93%). However, in IFN-α–experienced patients the response was lower (SVR 47%) [26].

**NS5A INHIBITORS**

Daclatasvir (BMS, formerly BMS-790052) is the first drug in this class. NS5A is not a viral enzyme but a protein with pleiotropic functions. Daclatasvir potently inhibits HCV replication across all genotypes. Results of a trial combining daclatasvir with the protease inhibitor asunaprevir (BMS, formerly

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<th>Table 1. Main Differential Characteristics of Direct-Acting Antivirals</th>
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<td><strong>Mechanism of inhibition</strong></td>
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BMS-650032) in HCV-1b, noncirrhotic, Japanese patients were presented at EASL [27]. An IFN-α–free regimen was chosen because recruited patients were prior null responders or intolerant to pegIFN-α/RBV. After 24 weeks of dual therapy, SVR12 rates ranged from 64% to 91%.

An all-oral combination of daclatasvir, sofosbuvir, and/or RBV demonstrated high end-of-treatment response in patients infected with HCV genotypes 1, 2, and 3 treated for 24 weeks [20]. Finally, an oral quad regimen including an NS5A inhibitor (GS-5885), a NS5B nonnucleoside polymerase inhibitor (tegobuvir, formerly GS-9190), and an NS3 protease inhibitor (GS-9451), along with RBV brought major attention [28]. Quadruple therapy for 24 weeks resulted in 80%–100% SVR4. Interpretation of these results should be cautioned by the short follow-up and the limited size of the study population. The regimen was generally well tolerated, although indirect hyperbilirubinemia developed in 60% of patients.

**PREDICTORS OF RESPONSE TO DAA**

Baseline predictors of treatment outcome using pegIFN-α/ RBV still play a role using DAAs. This is the case for serum HCV RNA, HCV genotype, liver fibrosis stage, and IL-28B alleles. Moreover, new predictors of treatment response have emerged using DAAs. A retrospective subanalysis in the PROVE-2 trial, examining IL-28B variants in noncirrhotic patients, showed similar SVR rates in patients carrying IL-28B CC alleles, either in the 12 individuals randomized to 12 weeks of triple therapy (SVR 100%) or in the 16 individuals who remained on pegIFN-α/RBV for another 12 weeks (SVR 94%) [29]. On the basis of these results, the CONCISE study is currently testing whether 12 weeks of triple therapy are enough for noncirrhotic, IL-28B CC, IFN-α–naive, or prior relapsers who attain RVR.

A retrospective analysis in the REALIZE trial showed that there is an inverse correlation between pretreatment IFN-γ inducible protein 10 (IP-10) levels and SVR rates using triple therapy with telaprevir [30]. Levels of IP-10 were associated with type of prior failure to dual therapy, being low in relapsers, intermediate in partial responders, and high in null responders. In fact, prior failure modality was a better predictor of response than any other host-related factor. Compared with IL-28B variants, levels of IP-10 were better predictors of SVR to triple therapy, although the combination of both factors, IL-28B and IP-10, gave the best prediction.

Rapid serum HCV RNA decay within the first days of triple therapy with telaprevir is universally seen regardless of treatment history (IFN-naïve or experienced patients) or prior failure modality (breakthrough, relapse, or partial response). With the exception of most early breakthroughs (within the first 12 weeks of therapy), very low HCV RNA levels are always attained by week 4 of therapy. However, all patients with HCV RNA >1000 IU/mL by week 4 or 12 of therapy experience breakthroughs [31]. Interestingly, early HCV RNA decline was as rapid in relapsers as in late breakthroughs. Failure to triple therapy may not be attributed to poor drug potency but to selection of already preexisting resistance mutations [32]. Different impairment on viral fitness caused by distinct drug resistance mutations could explain different lags in HCV rebounds (Figure 1). Based on this rationale, implementation of more strict futility rules may further prevent unnecessary costs and toxicities [33].

RBV exposure and, accordingly, RBV dosing have been so far considered critical for maximizing response to dual HCV therapy [34]. The benefit of RBV is recognized both in early phases, enhancing HCV RNA decay, and thereafter, reducing relapses. However, a substudy of telaprevir phase 3 trials has shown that RBV dose reductions, to as low as 600 mg daily, in order to temper anemia, do not compromise SVR rates [35]. No effect of tapering RBV was observed when comparing dose adjustments done within the first 4 weeks of triple therapy or later, or even if patients had detectable or undetectable HCV RNA at that time. However, permanent RBV discontinuation was associated with lower SVR. A similar subanalysis conducted in boceprevir studies showed that either tapering RBV or using erythropoietin for managing anemia resulted in similar SVR rates, regardless of sex, race, body weight, liver fibrosis stage, or IL-28B variants [36].

**RESISTANCE TO DAAs**

Before the arrival of DAAs, drug resistance was not a concern in HCV therapeutics. However, failure with selection of drug resistance mutations uniformly occurs in patients experiencing
virologic failure on DAA, nucleos(t)ide analogues being the only exception. Resistance is selected very rapidly [37], which justifies recommending early stopping time points for drug discontinuation (futility rules) if HCV replication still persists under drug pressure [38, 39].

Virologic failure and more rapid selection of resistance is intriguingly seen more frequently with almost all DAAs in patients infected with HCV subtype 1a vs subtype 1b. Although this finding was formerly attributed to differences at codon 155 in HCV-1 subtypes [40], other reasons must contribute to this lower therapeutic susceptibility of HCV-1a, including a lower response to IFN-α [41, 42].

The influence of baseline natural polymorphisms at positions involved in drug resistance within the HCV genome had been highlighted previously [43-48], but no studies had prospectively tested their clinical impact. The influence of HCV protease polymorphisms at codon 80 on response to simeprevir was specifically addressed in the ASPIRE trial [9]. Patients infected with HCV-1b responded the best (80% SVR), whereas the worst response was seen in HCV-1a patients harboring Q80K when treated with simeprevir 100 mg/day (22% SVR) [49]. This mutation reduces nearly 11-fold simeprevir susceptibility in vitro and is present in approximately 25% of HCV subtype 1a. Interestingly, use of the greatest dose of simeprevir in this trial (150 mg/day) somewhat overcame the harmful impact of Q80K [49]. In studies with other HCV protease inhibitors, such as ACH-1625, the impact of Q80K was mainly seen in HCV subtype 1a with unfavorable IL-28B gene polymorphisms [50]. Thus, it may be worth performing baseline drug resistance testing when some DAAs are considered for treating HCV-1a patients with unfavorable IL-28B alleles.

**IS THERE A RESERVOIR FOR HCV?**

In contrast with HIV, there is no integration of the HCV genomic material within infected cells during viral replication [47]. HCV RNA survives exclusively in the cytosol as part of a dynamic process of synthesis and release in newly encapsidated virions. The half-life of these HCV RNA molecules is short as cellular RNAses degrade them constantly. On the basis of this biological knowledge, a complete blocking of viral replication with drugs exerted during a minimum period of time should lead to eradication (Drusano’s rule) [51]. Accordingly, shorter treatment duration should be given to patients achieving undetectability more rapidly whereas longer therapy should be provided to slow responders [52].

Two recent observations with DAAs reported at the 2012 EASL meeting are challenging this view. The first is that the subset of patients with prior null response to IFN-α who received sofosbuvir/RBV all achieved undetectability rapidly and maintained complete viral suppression for 12 weeks. However, relapse occurred almost universally after treatment discontinuation [17]. The second observation is that very late relapses (beyond week 24 after ending treatment) have occasionally been reported using IFN-α-free combinations of DAAs [25].

Until recently, continued undetectable serum HCV RNA at week 24 after completion of treatment (SVR24) was considered to be a reflection of cure. However, recent studies have highlighted that most relapses occur within the first 12 weeks after discontinuation of therapy [52-54] and accordingly, regulatory authorities have decided that SVR12 is acceptable for demonstrating effectiveness in clinical trials. Altogether, these findings suggest that besides complete viral suppression for a minimum period with drugs, the immune system might be required to trigger definitive clearance of the remaining HCV RNA present within the cytosol of infected hepatocytes.

**Notes**

**Acknowledgements.** This work was funded in part by grants from Fundacion Investigacion y Educacion en SIDA (F-IES), the European Network of AIDS Trials (NEAT) and Red de Investigacion en SIDA (RIS, project RD06/0006). We would like to thank John McHutchison, Josep Mallolas and Christoph Sarrazin for helpful comments and sharing information presented at the conference.

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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