Chapter 6

HCV Treatment Failure in the Era of DAAs

Mohamed Hassany and Aisha Elsharkawy

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67149

Abstract

Hepatitis C virus (HCV) has six well-known genotypes in worldwide and has a very high genetic diversity. Introduction of DAAs leads to improvement of treatment results with SVR rates exceeding 95%. Development of HCV treatment resistance is a problematic issue that needs sufficient solutions. Many hosts, viral, and drug factors are implemented in the process of treatment resistance. Lack of clinical trials on treatment failure leads to lag in development of certain consensuses for retreatment.

Keywords: HCV-DAAs, viral resistance, treatment failure

1. Introduction

Chronic hepatitis C virus (HCV) infection is a major health problem all over the world. The global prevalence of viremic HCV infection was reestimated between 64 and 103 million patients [1]. Chronic HCV patients suffered a long time from the complications of their disease until the first discovery of interferon treatment. However, its modest response rate and the development of many adverse events were the major problem. Soon the dream seems to become true with the introduction of HCV direct acting antivirals (DAAs) in 2014. Their higher rates of response and minimally observed adverse events encourage more patients to go for treatment. In addition, patients with advanced fibrosis and cirrhosis find a new hope to stop the progression of their disease. Three classes of DAAs (protease inhibitors, NS5A inhibitors, and polymerase inhibitors) targeting three HCV enzymatic nonstructural proteins were approved for treatment in many countries [2]. Variability of treatment efficacy among patients makes it difficult to control the infection; while for some patients, weak antivirals and short-term treatments are sufficient, others require combination therapies with several highly active antivirals for longer durations [3].

Despite the high rates of virological cure achieved with these treatments, the infection is not eliminated from a substantial number of patients (1–15%, depending on the patient status and regimen used) [4]. Patients and researchers started to face the new problem of drug resistance. In this review, HCV treatment failure in the era of DAAs will be discussed in the context of factors affecting development of resistance, diagnosis, and management.

2. Treatment from interferon to DAAs

Hepatitis C virus (HCV) has six well-known genotypes in worldwide [5–10] with multiple subtypes (a, b, c, etc.). RNA sequence may vary by 35% between different genotypes. HCV has a very high genetic diversity and very high rate of replication (>10 trillion virions/day), and due to this replication rate, significant genetic errors occur and a continuous process of correction is already running to optimize the replication and sequencing of the virus genes; failure of error correction leads to the formation of genetic drifts [5]; these drifts are represented either in the form of genotypes or quasispecies. **Table 1** shows the difference among genotypes, subtypes, and quasispecies.

The presence of different HCV genotypes does not exhibit a major clinical implication on the natural history of the disease and its progression, yet it has a great influence on treatment outcome. The best results for treatment in the past era of pegylated interferon (PegIFN) and ribavirin (RBV) were achieved in genotypes 2, 3 (80–90%) with less favorable results in genotypes 1, 4 (40–50%) and intermediate results in genotypes 5, 6 (60–70%). Failure of treatment during this era had no satisfactory solutions rather than retreatment using the same regimen or changing the pegylated interferon type (between alpha 2a and alpha 2b) or even extending the treatment duration.

Introduction of the direct acting antivirals (DAAs) in the playground of HCV treatment represents a major challenge with the rising number of approved molecules and its coming followers in the pipe of production and approval as shown in **Table 2**, although the very high response to these drugs, which sometimes exceeds 95% yet its limited failure, represents a problematic issue.

DAAs permit to treat different categories of patients who could not be treated easily in the past due to the low efficacy and safety of pegylated interferon such as those with advanced liver disease (CHILD-PUGH B, C), autoimmune diseases, polymedicated patients, renal impairment, postorgan transplantation, etc. Implementation of larger groups to the treatment pipe leads to expulsion of more numbers of treatment failures asking for better solutions for retreatment.

Genotypes, subtypes	Quasispecies
Difference in RNA sequence	Mutation during replication
Major genetic differences	Minor genetic differences
Does not change	Continue to evolve over time

Table 1. Differences between genotypes, subtypes, and quasispecies.

Agent class	Generation	Compound	Phase of clinical development
NS3-4A protease inhibitors	First-wave First-generation	Telaprevir Boceprevir	Approved
	Second-wave First-generation	Simeprevir Paritaprevir/ritonavir	Approved
		Asunaprevir Vaniprevir Sovaprevir	In clinical development
	Second-generation	Grazoprevir	Approved
		ACH-2684	In clinical development
Nucleoside/nucleotide analogues	Nucleotide analogues	Sofosbuvir	Approved
		MK-3682 ACH-3422 AL-335	In clinical development
Nonnucleoside inhibitors	Palm domain I inhibitors	Dasabuvir	Approved
	Thumb domain I inhibitors	Beclabuvir	In clinical development
	Thumb domain II inhibitors	GS-9669	In clinical development
NS5A inhibitors	First-generation	Daclatasvir Ledipasvir Ombitasvir	Approved
	Second-generation	Elbasvir Velpatasvir	Approved
		ACH-3102	In clinical development

Table 2. DAAa pipeline current situation (April 2016).

All the previously mentioned molecules have different characteristics regarding the potency, genotype coverage, and barrier to resistance. **Table 3** shows the characteristics of DAAs molecules [6].

Different continental guidelines for HCV management describe different treatment regimens:

1. *PegIFN-based regimens* (e.g., PegIFN + RBV + Sofosbuvir, PegIFN+RBV + Simeprevir, PegIFN + RBV + Daclatasvir)
2. *PegIFN-free sofosbuvir-based regimens ± ribavirin* (Sofosbuvir + Daclatasvir, Sofosbuvir + Simeprevir, Sofosbuvir + Ledipasvir, Sofosbuvir + Velpatasvir)
3. *PegIFN-free Sofosbuvir-free regimens ± ribavirin* (Paritaprevir/r + Ombitasvir ± Dasabuvir, Grazoprevir + Elbasvir)

Drug group	Potency	Genotype coverage	Resistance barrier
NS3-4A protease inhibitors	+++	+++	++
NS5A inhibitors	+++	+++	++
Nucleoside/nucleotide analogues	+++	+++	+++
Nonnucleoside inhibitors	++	+	+

Table 3. Characteristics of DAAs molecules.

3. Definitions

The terms RAVs, RASs, resistant variants, and sensitive variants were recently used in clinical practice to describe the susceptibility to an administered DAA. Using these definitions paved the way to understand more about HCV treatment failure when using DAAs. Pawlotsky has described well these terms as mentioned below [4]:

3.1. Viral resistance

Positive selection of viral variants with reduced susceptibility to an administered DAA.

3.2. Resistance-associated variant (RAV)

It is often used to indifferently describe the amino acid substitutions that reduce the susceptibility of a virus to a drug or drug class or, alternatively, the viral variants with reduced susceptibility that carry these substitutions.

3.3. Resistance-associated substitutions (RASs)

The amino acid substitutions that confer resistance.

3.4. Resistant variants

The viral variants carrying these RASs and thereby have reduced susceptibility to the DAA.

3.5. Sensitive variants

Viral variants that do not contain amino acids that confer reduced susceptibility to the antiviral action of an HCV DAA (contain only the original wild-type amino acids of the viral strains).

3.6. Fitness-associated substitution(s)

Single amino acid changes that do not alter DAA susceptibility but increase the power of replication (fitness of the resistant variants).

Prior to therapy, multiple baseline HCV resistant-associated variants (RAVs) are already present but usually at a very low undetectable limit. After treatment with DAAs, a sharp decline of HCV viremia occurs within the first treatment days and a competition between sensitive variants and resistant variants will determine which of the following scenarios will be encountered after stoppage of the administered drug:

(1) The drugs success to eliminate both sensitive and resistant variants and the patient succeed to achieve sustained virologic response (SVR).

(2) The drug eliminates the HCV sensitive variants and rendering the resistant variants and after stoppage of treatment both resistant and sensitive variants are restored to the same baseline picture and continue to replicate.

(3) The drug eliminates the HCV sensitive variants and rendering the resistant variants and after stoppage of treatment the resistant variants replicate as a dominant virus.

4. Factors affecting the outcome and HCV resistance

Failure of treatment and development of resistance are a multifactorial process depending on host-related factors, virus-related factors, and drug-related factors as shown in **Figure 1**.

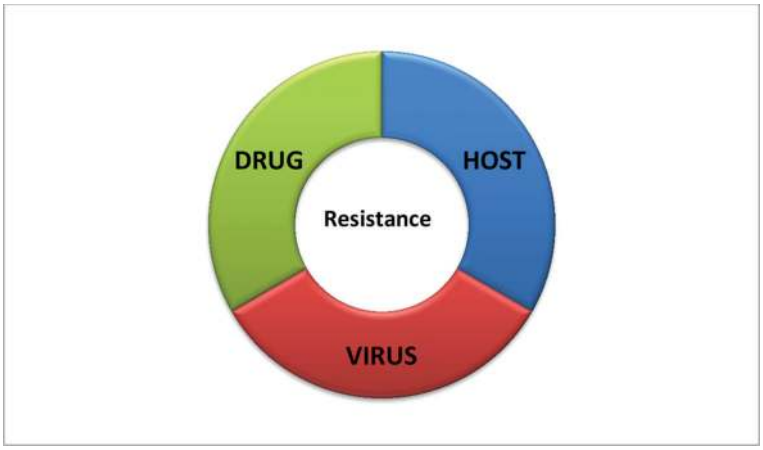

Figure 1. Factors affecting treatment outcome and development of resistance.

4.1. Host-related factors

Introduction of DAAs eliminates multiple host factors, which affect previous treatment with PegIFN and ribavirin, yet several host factors still persist:

(1) Adherence to therapy: achievement of the best drug response surely will be better in case of proper administration of the drug with its proper dose at regular times and respect of food relations as recommended by the manufacturer.

(2) HIV, post-organ transplantation and polymedicated patients: revision of the drug-drug interactions map is necessary in those patients to avoid the effect of other drugs in reducing the plasma level of anti-HCV drugs.

(3) Treatment status: most of clinical trials on DAAs showed mild better response in treatment naïve patients than those who previously failed treatment with PegIFN/RBV.

(4) Hepatic fibrosis stage: patients with advanced fibrosis stage remain the most difficult to treat group even under the umbrella of DAAs which showed a wide variable results in cirrhotics ranged between 33 and 100% [7]. Addition of ribavirin and prolonged treatment duration may offer the best chance for those patients in achieving sustained virological response.

4.2. Virus-related factors

(1) Genotype: treatment with PegIFN/RBV/Sofosbuvir represents the regimen that showed remarkable potency against all HCV genotypes. IFN-free regimens should be selected primarily based on genotype as we have pangenotypic regimens (Sofosbuvir + Velpatasvir ± RBV, Sofosbuvir + Daclatasvir ± RBV, Paritaprevir-ritonavir/Ombitasvir ± Dasabuvir ± RBV), regimens fit for all genotypes except genotype 3 (Sofosbuvir + Simeprevir ± RBV, Sofosbuvir + Ledipasvir ± RBV), and individualized regimens for genotypes 2-3-4 (Sofosbuvir + RBV).

(2) Baseline RAVs: The presence of baseline RAVs seems to be associated with variable degrees of treatment response. Zeuzem et al. [8], observed no significant difference in response in noncirrhotic genotype 1 patients treated with Sofosbuvir and ledipasvir between those with baseline RAVs and others without RAVs in different treatment status and durations (98% in RAVs group vs. 99% in no RAVs group in naïve patients treated for 8 weeks, 99% in RAVs group vs. 99% in no RAVs group in naïve patients treated for 12 weeks, 90% in RAVs group vs. 99% in no RAVs group in experienced patients treated for 12 weeks). However, a significant difference was observed in cirrhotic patients (88% in RAVs group vs. 100% in no RAVs group in naïve patients treated for 24 weeks, 87% in RAVs group vs. 100% in no RAVs group in experienced patients treated for 24 weeks) [4]. In C-EDGE study, Zeuzem et al. showed a great influence of baseline RAVs on treatment outcome in HCV GT1 patients treated with grazoprevir/elbasvir combined with very low SVR (22%) in GT1a patients with NS5A baseline RAVs > fivefolds potency loss [9]. No effect on SVR in genotype 1 HCV patients with or without cirrhosis with baseline RAVs treated with combination of ombitasvir, r-paritaprevir, and dasabuvir, with or without ribavirin, for 12 or 24 weeks in four phase three clinical trials [11]. When Sofosbuvir/Daclatasvir combination was used, the presence of NS5A baseline RAVs is associated with reduced rates of SVR in undertreated (too short duration, no ribavirin) patients with cirrhosis and genotype 3 infection

[12]. In addition, the presence of NS3 protease RAS Q80K was associated with a reduced rate of SVR in patients with HCV genotype 1a infection and cirrhosis, especially if they failed to respond to previous pegylated IFN–based treatment [13, 14].

4.3. Drugs-related factors

(1) Potency and genetic barrier: the ideal drug for HCV treatment is not only potent but also could keep this potency against all HCV strains until cure which is known as resistant barrier (**Table 3**).

(2) Drugs combinations: lessons learned from HIV and TB management of drug resistance, multiple drug resistance and extensive drug resistance, outlining the frame of HCV treatment. Using multiple potent drugs for ideal durations is the best way to achieve HCV cure.

(3) Posttreatment RAVs: emergence of posttreatment RAVs has a major impact on retreatment decision. NS3-4a RAVs appearing after treatment failure usually persists for short durations (12–18 months) posttreatment [10] while longer durations were observed in NS5A RAVs which sometimes persist for years [4, 15]. On the other hand, appearance of RAVs to Nucleoside/nucleotide analogues is extremely rare, and if happened, it is usually nonreplicative [16]. **Tables 4–6** show the different amino acid variants causing either resistance or cross-resistance in different DAAs classes.

Variant	Boceprevir	Telaprevir	Simeprevir	Paritaprevir
V36	R	R	-	S
T54	R	R	-	-
V55	R	-	-	S
V107	S	-	-	-
R155	R	R	R	S
A156	R	R	-	-
V158	S	-	-	-
D168	S	S	R	R
I/V 170	R	-	R	-
M175	S	-	-	-
I132	-	S	-	-
Q80	-	-	R	-
S122	-	-	R	-
Y56	-	-	-	R

Table 4. Resistance and cross-resistance in NS3-4A protease inhibitors.

Variant	Daclatasvir	Ledipasvir	Ombitasvir	Elbasvir	Velpatasvir
M/L/L28	R	-	-	-	-
P29	S	-	-	-	-
Q/R/L30	R	-	-	-	-
L31	R	R	-	R	-
P32	S	-	-	-	-
H/P58	R	-	-	-	-
E62	S	-	-	-	-
A92	S	-	-	-	-
Y93	R	R	R	R	R
M28	-	S	R	R	-
Q30	-	R	R	R	-
H58	-	S	S	-	-
M/L28	-	-	R	-	

Table 5. Resistance and cross-resistance in NS5A inhibitors.

Variant	Sofosbuvir	Dasabuvir
S282T	R	R
A421V	-	R
P495S/Q/L/A/T	-	R
C316Y/N	-	R
L419S	-	R
S368T	-	R
R422K	-	R
M414T/I/V/L	-	R
M423T/I/V/L	-	R
Y448C/H	-	R
I482L/V/T	-	R
G554D/S	-	R
A486/V/I/T/M	-	R
S556G	-	R
V494A	-	R
D559G	-	R

Table 6. Resistance in NS5B inhibitors.

http://dx.doi.org/10.5772/67149

5. Diagnosis of HCV RAVs

5.1. Diagnosis of resistance in clinical practice is conducted by two methods

(1) Phenotypic analysis: used to determine the optimum plasma concentration (effective concentration, EC_{50} EC_{90}) of the dug sufficient to inhibit the viral replication.

RAVs are typically associated with a change in the shape of the binding or interaction site of DAAs to HCV target proteins. RAVs harbor different levels of resistance due to different locations within the sites of interaction and different chemical structures of DAAs targeting the same site on the same HCV protein [3].

(2) Genotypic analysis (sequence analysis): used to detect the amino acids substitutes which cause drug resistance and treatment failure [17]. Clonal and deep sequencing technologies allow reliable detection of viral variants with a frequency down to 0.5–1% and commonly accepted level reached to 15% [18]. Generally, due to the high heterogeneity of HCV isolates and methodological restrictions all sequencing technologies may miss detection of RAVs due to nonamplification based on HCV RNA secondary structures, primer selection, and low frequencies within HCV quasispecies [3].

Resistance testing in clinical practice is not so easy, but it is actually very difficult. Limited number of well-equipped virological labs all over the world that can deal with these tests, experienced hands and the ability to interpret the results correctly, make testing for resistance a time and money consuming procedure and balancing the benefit versus the cost should be considered especially when dealing with large populations having different genotypes.

5.2. Timing of HCV resistance testing

Because of the above-mentioned limitations, resistance testing is not recommended before starting therapy with DAAs for the first time; especially in areas where HCV is highly endemic. Instead, trying to give patients the best chance of cure through using multiple drugs, adding ribavirin or prolongation of the treatment duration if needed may be a good decision; also testing at the time of treatment failure usually associated with high prevalence of quasispecies and RAVs.

On the other hand, testing of resistance before retreatment of patients who fail to achieve virological response with DAAs may have a benefit for the proper selection of the best DAA drug for retreatment [4].

6. Management of drug failure and drug resistance

Clear evidence is still not available about the best regimens, best duration, and best time for retreatment of patients with DAAs failures, yet European association for the study of the liver (EASL) [19] and American association for the study of the liver diseases (AASLD) [20] released their interim opinions for retreatment options.

EASL guidelines recommend that Sofosbuvir should be a cornerstone in any retreatment trial due to its high barrier to resistance, addition of 1 or 2 other DAAs preferably with no cross-resistance with the failed drug, addition of ribavirin if tolerable and prolongation of treatment duration to 24 weeks especially in cirrhotics.

AASLD guidelines using Sofosbuvir-based triple or quadruple DAAs with ribavirin if tolerable for 12–24 weeks in case of failure of Sofosbuvir-based dual regimen, RAVs testing prior to retreatment and the final treatment options is tailored based on its results.

Review of some recent published data in **Table 7** for retreatment of clinical trials appears to be insufficient to justify a competent guidelines, more data is needed to reach to the nearest figure to ideal. From these trials, we could choose one of the following models:

(1) The patients have no RAVs, so retreatment using the same failed regimen (or adding other drugs) could be allowed but add ribavirin if needed but not previously added and choose the ideal duration according to the patient status.

(2) The patient has RAVs to protease or polymerase inhibitors, which will disappear after few weeks or months, so we could choose either to wait until reset point or to use another family of DAAs like NS5A inhibitors plus sofosbuvir.

(3) The patient has RAVs to NS5A inhibitor drug without cross-resistance, so the failed drug could not be used but other drugs from the same family could be.

(4) The patient has RAVs to NS5A inhibitor at certain sites leading to resistance and cross-resistance, so the whole NS5A members from the same wave could not be used, shifting to different wave of the family or changing the whole group to protease inhibitors will be the best way.

	Description	Retreatment regimen	Results	RAVs impact
Wyles et al. [21]	51 GT1 patients with previous treatment failure 25 patients failed PegIFN/RBV/Sofosbuvir 20 patients failed Sofosbuvir/RBV 5 failed Sofosbuvir placebo/PegIFN/RBV 1 failed GS-0938 monotherapy	Sofosbuvir + Ledipasvir + Ribavirin for 12 weeks	50/51 (98%) SVR	NA
Forns et al. [22]	79 GT1 patients with previous treatment failure 66 patients failed PegIFN/RBV/protease inhibitor 12 patients intolerable to treatment with PegIFN/RBV/protease inhibitor	Grazoprevir + Elbasvir + Ribavirin for 12 weeks	76/79(96.2%) SVR	-100% in patients without baseline RAVs -91.2% with baseline NS3 RAVs -75% with baseline NS5A RAVs -66.7% in both NS3, NS5A RAVs

	Description	Retreatment regimen	Results	RAVs impact
Hézode et al. [23]	Real world data 16 GT1, 4 patients with previous treatment failure 13 patients failed PegIFN/ RBV/daclatsvir/asunaprevir 3 patients failed PegIFN/ RBV/daclatasvir	Sofosbuvir + Simeprevir for 12 weeks without ribavirin	14/16 (87.5%) SVR	Presence of Simeprevir RAVs (R155K and Q80K) had no effect on treatment outcome
Lawitz et al., C-SWIFT [24]	25 GT1 patients failed Grazoprevir + Elbasvir + Sofosbuvir for 4, 6, or 8 weeks	Grazoprevir + Elbasvir + Sofosbuvir + RBV for 12 weeks	100% SVR	No impact
Poordad et al. QUARTZ 1 [25]	22 GT1 patients with previous treatment failure to DAAs 14 patients to OBV/PTV/r + DSV 2 patients to OBV/PTV/r 2 patients to telaprevir 2 patients to SOF 1 patient to simeprevir/ samatasvir 1 patient to simeprevir + SOF	-OBV/PTV/r + DSV + SOF for 12 weeks in patients without cirrhosis -OBV/PTV/r + DSV + SOF + RBV for 12 weeks in GT1a patients without cirrhosis -OBV/PTV/r + DSV + SOF + RBV for 24 weeks in GT1a patients with cirrhosis	14/15 (93%) SVR 12 IN patients treated for 12 weeks, 7/7 (100 %) SVR 4 in patients received 24 weeks	No impact

Table 7. Review of recent data for retreatment.

7. Conclusion

HCV elimination is a worldwide goal; curing infection with oral drugs for short duration and minimal adverse events is going on. Appearance of resistance to DAAs is disappointing to the clinicians and the researchers yet choosing the proper treatment regimen initially leading to minimizing this problem. The ideal RAVs testing and interpretation lead to the best options to justify the retreatment regimen.

Author details

Mohamed Hassany[1] and Aisha Elsharkawy[2]*

*Address all correspondence to: a_m_sharkawy@yahoo.com

1 National Hepatology & Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt

2 Endemic Medicine and hepatogastroentrology, Faculty of Medicine, Cairo University, Cairo, Egypt

References

[1] Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61:S45–S57

[2] Bartenschlager R, Lohmann V, Penin F. The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. Nat Rev Microbiol. 2013;11:482–496.

[3] Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. J Hepatol. 2016 Feb;64(2):486–504.

[4] Pawlotsky J-M. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology. 2016 Jul;151(1):70–86.

[5] Farci P, Purcell RH. Clinical significance of hepatitis C virus genotypes and quasispecies. Semin Liver Dis. 2000 Jan;20(1):103–126.

[6] Asselah T, Marcellin P. Interferon free therapy with direct acting antivirals for HCV. Liver Int. 2013 Mar;33(Suppl 1):93–104.

[7] Majumdar A, Kitson MT, Roberts SK. Systematic review: current concepts and challenges for the direct-acting antiviral era in hepatitis C cirrhosis. Aliment Pharmacol Ther. 2016 Jun;43(12):1276–1292.

[8] Zeuzem S, Mizokami M, Pianko S, Mangia A, Han K, Martin R.et al. Prevalence of pretreatment NS5A resistance associated variants in genotype 1 patients across different regions using deep sequencing and effect on treatment outcome with LDV/SOF. AASLD. 2015. p. Abstract 91.

[9] Zeuzem S, Ghalib R, Reddy KR, Pockros PJ, Ben Ari Z, Zhao Y, et al. Grazoprevir-elbasvir combination therapy for treatment-naive cirrhotic and noncirrhotic patients with chronic hcv genotype 1, 4, or 6 infection: a randomized trial. Ann Intern Med. 2015 Apr 24;163(1):1–13.

[10] Lenz O, Verbinnen T, Fevery B, Tambuyzer L, Vijgen L, Peeters M, et al. Virology analyses of HCV isolates from genotype 1-infected patients treated with simeprevir plus peginterferon/ribavirin in Phase IIb/III studies. J Hepatol. 2015 May;62(5):1008–1014.

[11] Krishnan P, Tripathi R, Schnell G, et al. Resistance analysis of baseline and treatment-emergent variants in hepatitis C virus genotype 1 in the AVIATOR study with paritaprevir-ritonavir, ombitasvir, and dasabuvir. Antimicrob Agents Chemother. 2015;59:5445–5454.

[12] Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12- week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. Hepatology. 2015;61:1127–1135

[13] Kwo P, Gitlin N, Nahass N, et al. Simeprevir plus sofosbuvir (12 and 8 weeks) in HCV genotype 1-infected patients without cirrhosis: OPTIMIST-1, a Phase 3, randomized study. Hepatology. 2016 Aug;64(2):370–380.

[14] Lawitz E, Matusow G, DeJesus E, et al. Simeprevir plus sofosbuvir in patients with chronic hepatitis C virus genotype 1 infection and cirrhosis: a phase 3 study (OPTIMIST-2). Hepatology. 2016 Aug;64(2):360–369.

[15] McPhee F, Hernandez D, Yu F, Ueland J, Monikowski A, Carifa A, et al. Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and asunaprevir. Hepatology. 2013 Sep;58(3):902–911.

[16] Svarovskaia ES, Dvory-Sobol H, Parkin N, Hebner C, Gontcharova V, Martin R, et al. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. Clin Infect Dis. 2014 Dec 15;59(12):1666–1674.

[17] Fourati S, Pawlotsky J-M. Virologic tools for HCV drug resistance testing. Viruses. 2015 Dec;7(12):6346–6359.

[18] Dietz J, Schelhorn SE, Fitting D, Mihm U, Susser S, Welker MW, et al. Deep sequencing reveals mutagenic effects of ribavirin during monotherapy of hepatitis C virus genotype 1-infected patients. J Virol. 2013;87:6172–6181

[19] EASL Recommendations on Treatment of Hepatitis C 2015. J Hepatol. 2015 Apr 21;63(1):199–236.

[20] Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C Virus. Hepatology. 2015 Jun 25.

[21] Wyles D, Pockros P, Morelli G, Younes Z, Svarovskaia E, Yang JC, et al. Ledipasvir-sofosbuvir plus ribavirin for patients with genotype 1 hepatitis C virus previously treated in clinical trials of sofosbuvir regimens. Hepatology. 2015 Jun;61(6):1793–1797.

[22] Forns X, Gordon SC, Zuckerman E, Lawitz E, Calleja JL, Hofer H, et al. Grazoprevir and elbasvir plus ribavirin for chronic HCV genotype-1 infection after failure of combination therapy containing a direct-acting antiviral agent. J Hepatol. 2015 Sep;63(3):564–572.

[23] Hézode C, Chevaliez S, Scoazec G, Soulier A, Varaut A, Bouvier-Alias M, et al. Retreatment with sofosbuvir and simeprevir of patients with HCV GT1 or 4 who previously failed a daclatasvir-containing regimen. Hepatology. 2016 Jun;63(6):1809–1816.

[24] Lawitz EJ, Poordad F, Gutierrez J, Wells J, Landaverde C, Reiling J, et al. C-SWIFT retreatment final results: highly successful retreatment of GT1-infected patients with 12 weeks of elbasvir/grazoprevir plus sofosbuvir and ribavirin after failure of short-duration all-oral therapy. EASL 2016. Abstract SAT 148.

[25] Poordad F, Bennett M, Sepe TE, Cohen E, Reindollar RW, Everson G, et al. ombitasvir/paritaprevir/r, dasabuvir, and sofosbuvir treatment of patients with hcv genotype 1-infection who failed a prior course of daa therapy: the QUARTZ-I study. EASL 2016. Abstract SAT 156.