Mini Review

Mechanisms of virologic failure in hepatitis C and strategies for treatment failure

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Abbreviations: SVR, sustained virological response; RAV, resistance-associated variant; RAS, resistance-associated substitution; DAA, direct acting antiviral; PI, protease inhibitor; PR, pegylated IFN-alpha plus ribavirin; GT, genotype

Introduction

Up to 170 million people worldwide may be infected with the hepatitis C virus (HCV). HCV infection significantly contributes to morbidity and mortality rates worldwide. Chronic liver injury induced by HCV may produce hepatocellular injury and fibrosis with progression to cirrhosis, potentially resulting in portal hypertension, liver cancer, and death.[1,2] Sustained virological response (SVR) rates have markedly increased since the approval of direct acting antiviral (DAA) therapies. While individual DAAs have a low barrier to resistance, most patients may be successfully treated using DAA combination therapy. However, DAAs are vulnerable to drug resistance, and resistance-associated variants (RAVs) may naturally occur prior to DAA therapy or may emerge following drug exposure. While most RAVs are quickly lost in the absence of DAAs, compensatory mutations may reinforce fitness.[3] In spite of the potent, and highly efficient, novel treatment regimens, response data outside of clinical trials suggest that treatments will fail in approximately 10% to 15% of patients.[4] HCV kinetics in antiviral treatments are typical biphasic. The first-phase HCV RNA decline is rapid and occurs due to the direct inhibitory effect of the drug(s) on viral replication. The slower second-phase HCV RNA decline results from the progressive cure of infected cells, as a consequence of degradation of non-replicating viral RNAs by effectors of the innate immune system.[5]

The absolute amount of each viral population present in the quasispecies at baseline evolves following individual kinetics that depend on the starting amount of the viral population, susceptibility to the antiviral action of the drugs and fitness in the presence of the drugs. Sensitive viral populations are rapidly eliminated following a typical biphasic decline if treatment duration is sufficient. In contrast, resistant variants, which are only partly or not inhibited, slowly decrease, remain at the same level or outgrow. Therefore, some of these variants are still present in the liver when treatment is stopped, even though they were undetectable in peripheral blood samples during the treatment. After treatment is withdrawn, these resistant variants start replicating again, eventually acquire mutations that improve their fitness, and propagate in the liver, ultimately causing virologic relapse. At treatment failure (breakthrough or relapse), if adherence and treatment duration have been appropriate, most variants are resistant to one or several of the drugs administered. After treatment, some variants (such as those resistant to NS3-4A protease inhibitors (PI)) disappear within a few weeks to months. Their replacement by the wild-type virus may result from its persistence in the liver if therapy was not sufficient long to remove it or due to spontaneous mutations in resistant viruses that randomly revert to the wild-type if the original wild-type virus had been cleared during therapy. Other variants (such as those resistant to NS5A inhibitors) persist for years after treatment failure, because they are naturally fitter than wild-type viruses or are unable to revert to the wild-type for genetic reasons.[6]

Mechanism of the virologic failure

Factors associated with failed interferon (IFN) therapy have been a major topic of research in the last 2 decades. Race, comorbidities, interleukin 28B (IL28B) status, viral genotype (GT)/subtype, and degree of liver fibrosis have been identified as major factors. When patients fail potent DAA regimens, the reasons for failure appear to be distinct from the IFN era, although some may retain some importance as clinicians begin to report their experience. Viral, host and pharmacological factors all contribute to treatment failure in the DAA era. The presence of RAVs may have an impact on therapy during retreatment with DAAs. Salvage therapy in DAA experienced patients is still an area of emerging research, and each patient's treatment plan needs to be individualized.[7]

Viral factors

It rapidly became clear that viral resistance would be a major problem during long-term anti-retroviral therapy. Combination of drugs with different viral targets and no cross-resistance now represent a valuable option for preventing human immunodeficiency virus (HIV) resistance in the long term. HCV shares many properties with HIV: it is a highly variable virus with a quasispecies distribution, large viral populations, and very rapid turnover. However, unlike HIV, the HCV replicative cycle is exclusively cytoplasmic and there is no host genome integration or episomal persistence in infected cells. Therefore, HCV infection is inherently curable. Nonetheless, multi-drug-resistant viruses have emerged in some patients after several years of combination therapy. These viruses escape the antiviral effects of all available drugs, leading to treatment failure, disease progression, and death. Resistance is still the principal challenge in anti-HCV therapy.[8]

The history of waves of treatment options for HIV-1 infection revives for HCV. There are still treatment protocols that await further exploration. Standard combination therapies may now be applied in modified ways that have the potential to further increase SVR rates. The new design includes two-phase split treatments, the use of broad spectrum antiviral agents based on stimulation of innate immune response, or lethal mutagens that achieve virus extinction through an excess of deleterious mutations, the introductions of drugs that target cellular functions needed by the virus, or combinations of immunotherapy and pharmacology. Furthermore, when a mutagenic agent is applied to therapy, the sequential inhibitor-mutagen combination may have an advantage over the corresponding combination.[9] The high error rate of the HCV polymerase coupled with virion production, which is 100-fold higher than that of HIV, results in quasi-species that preexist within an infected individual before treatment initiation. While the selection of preexisting viruses with reduced susceptibility to administered drugs plays a role in treatment failure, the full clinical impact of HCV drug resistance, its implications for retreatment, and the potential role of baseline resistance testing remain critical research and clinical questions.[10]

It is also important to note that some amino acid substitutions may have an impact on resistance to several inhibitors. Therefore, memory can also affect response to inhibitors other than those used in the first treatment. Memory has also been documented with HIV-1 *in vivo*, and it may contribute to treatment failure when a patient is treated for a second time with a drug that was already administered in a previous treatment.[9] Resistance-associated mutants may arise at any time prior to or during therapy. Most variants have lower fitness loss than that of the wild type and are quickly lost; however in the worst case, secondary mutations may compensate for fitness loss and allow the variant to remain even in the absence of the drug. The single amino acid substitutions and some double amino acid substitutions (Q54H-Y93H) observed in GT 1b confer minimal resistance; however, some double amino acid substitutions in GT 1b, such as L31V-Y93H, confer high levels of resistance. Some variants with double amino acid substitutions (such as Q30R-H58D) also confer high levels of resistance. This may, at least partially, explain why viral breakthrough is more commonly observed in patients infected with GT 1a than GT 1b.[11]

The RAVs of HCV have been attributed to a high viral replication rate, high error rate of viral RNA-dependent RNA polymerase, and the lack of overlapping reading frames in the HCV non-structural region. During HCV infection, the wild-type of virus predominates but when several isolates in the HCV-quasispecies can undergo mutations and then confer resistance to DAAs by direct or indirect effects. It is also known that SVRs vary by subtype of GT 1, showing better SVR in genotype 1b than GT 1a. This variation is attributed to lower genetic barriers to resistant variants at key sites on HCV NS3 protease in patients with GT 1a compared with patients with 1b. The relapse rate is higher in patients with baseline NS5A RAVs than in those with no RAVs. Viral factors, such as the baseline viral load, have been implicated in the virologic non-response.[7]

HCV resistance to DAAs is influenced by three major parameters.

a. The genetic barrier to resistance, related to the number and type of nucleotide substitutions required to naturally acquire a RAS from the wild-type sequence, and the number and type of RASs required for a viral variant to acquire full resistance to the drug. The genetic barrier to resistance varies with the drug class, specific drug and the HCV GT/subtype. It affects the likelihood that resistant viruses are generated during replication;

b. The fitness of resistant viral populations, which is independent of the level of resistant conferred by RASs. It affects the likelihood that generated resistant viruses persist in minor or major populations;

c. Drug exposure influences the ability of the drug to inhibit replication of resistant variants.[6]

The genetic barrier is the threshold at which a virus will mutate and replicate under selective pressure from a drug. A viral population with a low genetic barrier to resistance may have a pre-existing mutation, which does not have an impact on viral fitness, but rapidly emerges during therapy. A viral population with a high genetic barrier may require either a primary mutation that severely impacts viral fitness or several mutations in order to develop drug resistance. In addition, cross-resistance, in which a resistant mutation results in decreased sensitivity to a specific drug or class of DAA, may occur and influence future treatment options. An additional concern is the persistence of resistant mutations or RAVs. Theoretically, when HCV therapy is discontinued in patients who do not clear the virus, the dominant population of the virus that re-emerge is the fitter or wild-type virus, because resistant HCV variants replicates less efficiently.[12] The presence of new variants in post-treatment samples may also represent previously undetected minority variants selected by the treatment or compartmentalized strains within different regions of the liver, lymphocytes, or the central nervous system.[13]

The NS proteins of HCV are the primary targets of DAAs that are either approved or in advanced development;

I. NS3/4A PI binds to the active site of NS3/4A protease;

II. NS5A inhibitors interact with domain 1 of the NS5A dimer, although the exact mechanism underlying the inhibition of NS5A has not yet been fully elucidated; nucleos(t)ide analog NS5B polymerase inhibitors are incorporated into the nascent RNA chain and result in chain termination by compromising binding of the next incoming nucleotide;

III. Non-nucleotide NS5B polymerase inhibitors interact with either the thumb 1, thumb 2, palm 1, or palm 2 domain of NS5B and inhibit polymerase activity by allosteric mechanisms of action.[10]

When a DAA is administered, the positive selection of viral variants with reduced susceptibility to this drug defines "viral resistance". Complete inhibition of DAA-sensitive wild-type viruses opens the replication space, thereby allowing variants with reduced susceptibility to rapid outgrow. Additional, so-called "compensatory" or "secondary" amino acid substitutions, or "fitness-associated substitutions", either naturally present or acquired by mutations during replication of the resistant virus upon drug administration, may increase the fitness of resistant variants, leading to their rapid outgrowth during (breakthrough) or after treatment (relapse), thereby influencing their post-treatment persistence.[6]

Some RAVs detected in treatment-naïve patients appear to be associated with the patient's IFNL3 GT, which might indicate selection due to innate immune responses in these patients.[14]

NS5B nucleotides have high barriers to the emergence of RAVs. NS3 PIs, NS5B non-nucleosides, and NS5A inhibitors have lower barrier to resistance.[15] NS5B is a key enzyme in HCV RNA synthesis, and its active site is highly-conserved. As a result, drugs targeting this site have pan-genotyppic activity and a high barrier to resistance. The most common mutation identified at the active site of NS5B is S282T,

which may be a pre existing mutation or emerge during treatment. However, this variant has diminished fitness and replicates at a much lower rate than the wild-type virus. These mutations occur more commonly in GT 1a than in GT 1b.

Resistant strains appear to persist after therapy is discontinued, implying a higher degree of viral fitness.[12] GT 3 is the most virulent strain leading to an increased risk of cirrhosis and hepatocellular carcinoma and appears to be the most difficult to eradicate with currently available DAAs.[15] Combination therapy with DAAs exhibiting a high barrier to resistance that target different segments of the HCV life cycle are associated with a low risk of the emergence of resistance and improved efficacy related to curing HCV infection.[12]

Host factors

Host cytokines and the innate immune response play an important role in regulating HCV. A persistent HCV infection results from inefficient innate and adaptive immunity. Innate immune responses regulate adaptive immune responses via direct interactions and by means of the exchange of signals between immune cells. HCV disturbs the activation of innate immune responses. The NS proteins of HCV, particularly NS3/4A, have been found to interfere with type I IFN induction pathways. IFNs are the first line of defense against viruses and act directly on viral replication and indirectly via the activation of immune responses. Genetic polymorphisms, such as IL28B, regulate cytokine production, which may be associated with the variation in responses to a virus. The role of polymorphism in tumor necrosis factor-α, transforming growth factor β1 and IL-10 have been demonstrated in chronic hepatitis C. Single nucleotide polymorphisms (SNPs) in a linkage disequilibrium block encompassing the 2 IFN-κ gene on chromosome 19 have been strongly linked to the response of PEG-IFN and RBV in many GTs of HCV. Genome-wide associated studies revealed that SNPs near the IL28B locus were associated with treatment responses in patients with GT 1 hepatitis C. Slightly higher SVR rates have been reported in patients with the favorable IL28B genotype CC than in those with TT in IFN-free DAA trials. Other host factors that contribute to the antiviral non-response include age, sex, race, alcohol use, obesity, insulin resistance, hepatic steatosis, and vitamin D and vitamin B12 deficiencies.[16] The CC genotype may increase early viral suppression by enhancing responsiveness to endogenous IFNs that are released as a result of the rapid anti-viral activity of DAA therapy.[17]

Since the progression of liver disease is essentially due to the local immune responses targeting infected hepatocytes, and as triple-drug treatment failure has not been associated with ALT flares, the selection of PI-resistant variants that have been present for years as minor viral populations may not lead to a shift in immune-dominance resulting in strong intrahepatic cellular immune responses and the associated production of proinflammatory cytokines, which accelerate liver disease progression.[8] Strong NS3-specific T cell immune responses at the baseline may predict a positive outcome for DAA-based therapy, and the presence of pre-existent existing resistance mutations does not play a significant role in the outcome of anti-HCV combined therapy.[18] NS3/4A HCV PIs achieve high antiviral potency by blocking HCV poly-protein cleavage and may also neutralize HCV NS3 protease-mediated interference with the innate immune system. Through this mechanism, HCV NS3/4A PIs reverse the HCV NS3 protein's capacity to block intracellular signal-transduction pathways for endogenous IFN production in vitro and may also do so *in vivo*.[19]

Another selective force that continues to shape HCV diversity throughout the course of infection is the host human leukocyte antigen (HLA)-restricted immune response and the presence of T-cell receptors (TCRs) specific to these epitopes. HCV-specific T cells are stimulated by the presentation of processed viral epitopes in the context of the HLA molecules. Substitutions in viral epitopes may alter HLA binding or their recognition by TCRs and result in the emergence of escape mutation. Therefore, the selection of HCV sequences targeted by the immune response is dependent on the HLA and T-cell repertoires of the host. Mutations within or flanking HLA-restricted HCV epitopes allow the virus to evade host immune responses. However, variability within the immuno-dominant cytotoxic T lymphocyte (CTL) epitopes of the NS3 protease is limited by viral fitness. Not all mutations at critical CTL-recognized epitopes are preserved during HCV infection. Some mutations may reduce protease activity and RNA replication (viral fitness). Therefore, viral fitness can limit the variability of HCV within immunological epitopes. This helps to explain why certain immunological escape variants never appear as a major viral quasispecies during infection.[18]

IFN lambda may play a larger role in treatment of HCV. IFNs may continue to play a useful role in treating or conditioning patients with DAA resistance because their broad antiviral activities contribute to the clearance of resistant strains, thereby improving the chance of successful re-treatment with DAA therapy. Although treatment guidelines for patients with emergent RAVs have not been fully established, DAA therapy should be discontinued in such patients.[14]

Persistent infections with HCV may depend on viral inhibition of host defenses. The HCV NS3/4A serine protease blocks the phosphorylation and effector action of IFN regulatory factor-3 (IRF-3), a key cellular antiviral signaling molecule. The disruption of NS3/4A protease function by a mutation or a ketoamide peptidomimetic inhibitor relieved this blockade and restored IRF-3 phosphorylation after a cellular challenge with an unrelated virus. The NS3/4A protease represents a dual therapeutic target, an inhibitor of which may both block viral replication and restore the IRF-3 control of HCV infection. HCV persistence is facilitated by the ability of the virus to incorporate adaptive mutations and to replicate as a population of genetically distinct quasispecies, but is likely to result from specific disruption of host immune responses by HCV proteins. IRFs are key transcription factors that initiate this cellular antiviral state. Disruption of the IRF-3 pathway by one or more HCV proteins blocks expression of IRF-3-activeated genes. NS3/4A serine protease activity is required for the blockade of the IRF-3 pathway and that this blockade may be reversed by antiviral inhibition of protease activity. IRF-3 induces the expression of a number of cellular genes including type 1 IFNs, which contribute to and further amplify the antiviral responses by including hundreds of IFN-stimulated genes. The inhibition of IRF-3 activation may not only promote viral persistence after an initial infection, but also reduce the effectiveness of IFN therapies, because many IFN-stimulated genes contain IRF-3 target sites within their promoter/enhancer regions. Candidate antivirals that target HCV protease while blocking viral replication by interfering with poly-protein processing may also restore the responsiveness of the IRF-3 pathway.[20]

Patients with compensated cirrhosis are expected to achieve SVR rates similar to those who do not have cirrhosis. The most significant population requiring new therapies is GT 3 treatment-experienced cirrhotic patients, for whom optimal therapy remains unclear.[6] Virologic failure during dual oral therapy may develop more frequently among women than among men. The TT allele was detected more frequently among female patients than male patients.[21] An elevated body mass index and GT1a disease have emerged as the most important factors limiting treatment success in the general

population.[15] Markedly higher failure rates are expected when these therapies are used in more difficult-to-treat populations, such as patients with unfavorable genetic markers of IFN-responsiveness and specific subgroups such as African Americans and null responders to prior therapy with pegylated IFN(PEG-IFN)-α and ribavirin (RBV), or patients who have not yet been included in clinical trials, including patients with advanced liver disease, liver transplant recipients, HIV-co-infected individuals, hemodialysis patients, or immunosuppressed patients.[8]

Altered pharmacokinetics

Hepatic metabolism is key to multiple DAA drugs. If hepatic metabolism is compromised in patients with extensive fibrosis or cirrhosis, it may disturb drug metabolism and lead to toxicity. Reduced metabolism from drug-drug interaction (DDI) or poor hepatic or renal function have an impact on the intracellular concentrations of active drug. Drugs that include these pathways may decrease the efficacy of Sofosbuvir (SOF). Similar to SOF, Ledipasvir (LDV) marginally affects the cytochrome P (CYP) enzyme. LDV is also a substrate of pg-P; therefore, drugs that induce the transporter protein pathway also need to be avoided. The key to achieving adequate serum levels of LDV and GS-5816 is an acid-rich environment in the gut.[7]

Porto-systemic shunting and cirrhosis

The liver plays a central role in the absorption, distribution, metabolism, and excretion of drugs and the drug pharmacokinetics, and these may be influenced by the presence of cirrhosis. The mechanism underlying the development of resistance in patients with advance liver fibrosis currently remains unclear. Decreased hepatic metabolism and porto-systemic shunting have been associated with changes in the pharmacokinetics of drugs.[7]

Strategy for treatment failure

The earlier HCV is eradicated, the greater the long-term benefit and the long-term savings are obtained.[15] Agents providing the greatest viral suppression leading to an extended rapid virologic response (RVR) may be preferable as an initial early induction approach. In order to achieve SVR, it is necessary to

i. Shut down virus production and achieve a rapid initial decline in circulating HCV RNA;

ii. Maintain viral inhibition throughout the treatment; and

iii. Induce a significant, slower second phase decline in HCV RNA, leading to the gradual clearance of HCV-infected liver cells through cell death or, more often, HCV removal. It has been hypothesized that the second-phase decline is driven by the patient's adaptive immune response in the context of the sustained inhibition of virus production. SVR may only be achieved if the second-phase decline is gradual and treatment lasts sufficiently long to ensure that all infected cells are cleared or cured.

Nevertheless, there are no objective findings to support this hypothesis. The addition of RBV enhances the second-phase decline induced by PEG-FN-α, thereby accelerating the clearance or cure of infected cells through unknown molecular mechanisms. Another hypothesis is that the restoration of intracellular innate immune response by viral inhibition plays an important role in the clearance of residual HCV genomes from these cells.[8] These patients may be treated with PEG-IFNα plus RBV (PR) therapy as a lead-in therapy to restore wild type frequencies prior to attempting further DAA therapy.[14]

Early virologic clearance by induction treatment with natural IFN-beta for 24 weeks before the beginning of PI with PR induced the restoration of innate immune responses linked to adaptive immune responses, resulting in SVR. Induction treatments associated with reductions in HCV.RNA levels before the beginning of triple therapy with PIs with PR may be used to treat difficult-to-cure chronic hepatitis C (CHC) patients with GT 1b and a high viral load. The clearance of HCV may lead to the restoration of innate and adaptive immune responses. However, many challenges are associated with achieving a solution to the induction of persistent viral suppression linked to the restoration of innate immune responses resulting in SVR. Furthermore, IFNs have no resistance to HCV, which is different to DAAs. The presence of RAVs to NS5A inhibitors does not attenuate the efficacy of NS3 inhibitors or PR and the combination of PI and PR may represent an alternative treatment option against Y93H RAV to combination therapy with DAAs.[16]

In order to maximize the anti-HCV response and minimize resistance, combination therapy, similar to current HIV treatment, may be used to enhance the resistance barrier. Regimens containing less potent drugs with a lower barrier to resistance generally require 3 or more drugs to be combined in order to prevent viral breakthrough and relapse due to the emergence of multidrug resistant subtypes.[12] The selection of RAVs against single agents administered to patients chronically infected with HCV necessitates that DAAs targeting multiple viral proteins be developed to overcome failure resulting from the emergence of resistance.[22]

GT 3 patients with cirrhosis are still difficult to cure. RBV is needed in order to increase SVR rates. Long-term therapy, >16 or 24 weeks, is required even when using a combination of SOF and an NS5A inhibitor. The emergence of RAVs can compromise salvage therapy, but therapy is little available information to date on rescue therapy.[15] AASLD guidelines suggest that treatment resistance testing need to be performed prior to re-treatment in patients for whom prior NS5A therapy had failed; however no specific recommendations have been provided. Retreatment with SOF plus daclatasvir (DCV) reperesent a potential option in non-cirrhotic GT3 patients who fail SOF and RBV. The best current salvage therapy in those who tolerate IFN is the combination of SOF, PEG-IFN, and RBV. Very limited information is available on rescue therapy with SOF and DCV; however, this combination may also have potential efficacy in patients harboring NS5A RAVs.[14]

The triple combination of SOF/velpatasvir plus GS-9857 showed very high efficiency in GT1 patients who had prior DAA treatment.[15] SOF/DCV and SOF/LDV achieved the successful re-treatment of patients who failed to respond to PI-based treatments with emerging NS3 mutations. Since SOF-resistant variants have poorly fitness, they rapidly disappear after treatment withdrawal in the small number of patients from whom they were selected NS5A RASs have an impact on SVR to SOF/LDV in patients infected with GT 1a, particularly those with cirrhosis and/or who failed prior PEG-IFN-based treatment. RBVs appear to prevent the effect of pre-existing NS5A RASs.[23] Therefore, high barrier to resistance of SOF coupled with the lack of cross-resistance between NS5A inhibitors and PIs provide a rationale for retreating NS5A-containig regimen failures with a combination of SOF and a PI. Recent international guidelines recommended that patients infected with GTs 1 or 4 who have failed treatment on a DCV plus PR regimen should be retreated with a combination of SOF and SIM, generally with RBV.[24]

Combination therapy with DAAs exhibiting a high barrier to resistance that target different segments of the HCV life cycle are

associated with a low risk of emergence of resistance and improved efficacy in curing HCV infection.[12] Triple regimens with non-overlapping mechanisms are expected to have high efficacy because the impact of individual RAVs are diminished.[7]

ASTRAL 1 investigated the combination of SOF and velpatasvir for the treatment of patients with hepatitis C GT 1, 2, 4, 5, and 6, and ASTRAL 2 compared the fixed-dose combination of SOF and GS-5816 with the combination of SOF and RBV in patients with GT 2.[25]

Baseline NS5A RASs do not influence the results of therapy with SOF/velpatasvir in non-cirrhotic and compensated cirrhotic patients, with the notable exception of those infected with GT 3. The addition of RBV appears to prevent the negative impact of pre-existing NS5A RASs better than prolongation of therapy to 24 weeks without RBV. SOF plus grazoprevir/elbasvir were retreated for 12 weeks with the same combination. Current guidelines already recommend the addition of RBV to 12 weeks of SOF/LDV, SOF plus daclatasvir or SOF pus simeprevir, or to prolong therapy to 24 weeks in order to reduce the rate of failure.[6] Patients treated in the FISSION, POSITRON, FUSION studies with GT 2 or 3 who failed to achieve SVR 12 were offered retreatment with 12 weeks of SOF plus PR or 24 weeks of SOF plus RBV.[7] Treatment need to be reinforced by the addition of RBV and /or by extending the duration of therapy. Although the field of HCV treatment is moving rapidly away from IFN-based regimens, a short course of highly effective IFN-containing treatment still have a role in subpopulations without better options. SOF plus PR for 12 weeks need to continue to be considered as a treatment option for eligible patients with GT 3 HCV. The use of SOF and RBV for 24 weeks represents an option for patients who cannot or are unwilling to take IFN.[26]

There is currently no FDA-approved regimen for prior all-oral DAA treatment failures. Based on available data, SOF appears to be the cornerstone of salvage therapy, even when patients previously failed SOF. In patients with GT 1, SOF may be combined with PEG-IFN/RBV, RBV alone, LDV, or LDV and RBV. Treatment duration vary from 8 to 24 weeks. The only option currently available for GT 2 or 3 failures is a longer duration of SOF and RBV or the addition of PEG-IFN to the regimen.[7] Drug combinations are sufficient to ultimately suppress the emergence of virally fit drug-resistant variants. Addition of PR to daclatasvir and asunaprevir as rescue or intensification therapy resulted in a cure for 33 % of patients who experienced viral breakthrough to daclatasvir and asunaprevir. Restoration of innate and/or adaptive immunity occurred after clearance of the virus from serum, thus enabling these patients to subsequently respond to quadruple therapy.[26]

The combination of grazoprevir and elbasvir is more effective at blocking the emergence of resistance. The combination of grazoprevir, an NS3/4A inhibitor, and elbasvir, an NS5A inhibitor, was found to potently inhibits HCVRNA synthesis, with no evidence of antagonism, and presented a high genetic barrier to resistance.[22] Grazoprevir-elbasvir achieved high SVR12 rates in treatment-naïve cirrhotic and noncirrhotic patients with GT 1, 4, or 6. Grazoiprevir retains strong activity against RAVs commonly detected after failed therapy with first-generation PIs.[27] A combination of elbasvir/grazoprevir plus SOF and RBV in patients who failed prior short-term treatment is developing. The drug was 100 % effective in all GTs. An 8 week combination of grazoprevir plus M 8408 for GT1, GT2 and GT3 patients is also developing.[15] NS5A RASs had a marked impact on response to 12 weeks of grazeprevir/elbasvir without RBV in all GT 1a patients and in GT1b prior non-responders. This effect was found to disappears if RBV is added and the treatment was prolonged to 16 or 18 weeks.[6]

Novel nucleotide analogues, such as MK-3682 or ACH-3422, may play roles in salvage therapy.[7] A pangenotypic NS3/4A PI, ABT-493 plus a pangenotyppic NS5A inhibitor ABT530 is developing. This combination is potent against common NS3 and NS5A variants and offers once-daily oral dosing with minimal renal excretion. Nucleos(t)ide inhibitors exhibit broad activities against different HCV GTs, have a high resistance barrier, and function by mimicking polymerase nucleotide substrates that are incorporated into the nascent RNA chain and result in chain termination.[15]

DDIs with HIV anti-retrovirals represent the most important unique consideration in treating HCV patients with HIV co-infection. The most prevalent DDIs in co-infected population involve the CYP 450 system (particularly the isozyme 3A4) or more complex multiple drug interactions that may lead to increased levels of TDF and potentially increase the risk of TDF-associated renal injury.[28] An effective prophylactic vaccine will remain the only viable option.[8]

Next generation regimens-The future of HCV therapy

The treatment of chronic HCV has undergone a recent phase of rapid evolution. Three IFN-free DAA regimens have been approved for the treatment of HCV GT 1. Within the next few years, continued development of novel agents and combinations is expected, with the aim of coming closer to the ideal regimen.[29] (1)phase III trials: combination of Daclatasvir, Asnaprevir and Beclabuvir-; Daclatasvir with SOF, (2) phase II trials: Grazoprevir(MK-5172) with Elbasvir(MK-8742); combination of Grazoprevir, Elbasvir and RBV; combination of Grazoprevir, Elbasvir and SOF; GS-5816(pan-genotypic NS5A inhibitor) with SOF; ACH-3102(NS5A inhibitor) with SOF, (3) phase I trial: ABT-493(PI) with ABT-530; MK3682(formally IDX21437; NS5B polymerase inhibitor) in combination with grazoprevir/elbasvir or grazoprevir/MK8408 (preclinical pan-genotypic NS5A inhibitor), (4) preclinical agents: MK-8408 in combination with other antivirals (grazoprevir et al), (5) host targeting agents (HTAs): MiR-122 (miR-122; HCV is protected within the hepatocyte by an abundant liver specific microRNA) inhibitors with DAA therapy; Miravirsen (miR-122 inhibitor); RG-101 (miR-122 inhibitor).[29]

Among GT patients with cirrhosis, the extension of treatment duration to 16 weeks is recommended for treatment-naïve and treatment experienced patients with cirrhosis.[30] DDIs are major consideration in deciding on the appropriate HCV DAA regimen in HIV co-infection; some patients require a switch in antiretroviral therapy. The race to develop the optimal regimen is far from over. Vigorous development plans continue to emerge. There continue to be hope of finding a pan-genotypic regimen that deliver high rates of SVR across all GTs and populations.[28]

Conclusion

SVR rates have increased dramatically after the approval of DAAs therapies. Despite the potent, and high efficiency, new treatment regimens, response data outside clinical trials suggest that treatment for around 10% to 15% of patients will fail. Viral, host and pharmacological factors contribute to treatment failure to achieve a SVR. It rapidly became clear that viral resistance would be a major problem during antiviral therapy. HCV escaped the antiviral effects of available drugs, leading to treatment failure, disease progression, and death. Resistance is still the principal challenge in anti-HCV therapy. Viruses resistant to NS3/4A PI disappear within a few weeks to months, whereas NS5A inhibitor resistant viruses persists for years, impairing results of re-treatment. Host cytokine and the innate immune response play an important role in regulating HCV. It is apparent that combination therapy with DAAs exhibiting a high

barrier to resistance that target different segments of the HCV life cycle will be associated with a low risk of emergence of resistance and improved efficacy related to curing HCV infection. Salvage therapy is still an area of emerging research, and each patient's treatment plan should be individualized.

Conflicts of interest

The authors declare there is no conflict of interests.

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