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*Review*

# Antivirals against HCV infection: the story thus far

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## Abstract

Remarkable scientific breakthroughs have been made in the stride towards the development of potent and tolerable hepatitis C regimens within the last three decades. Earlier approaches involved the use of pegylated interferon alfa and ribavirin as standard-of-care treatment. Treating genotype 1a infection with this regimen which was at that time considered the gold standard for hepatitis C virus therapy was rife with challenges; safety and toxicity issues necessitated a rigorous quest for alternative regimens. Deeper understanding of the pathogenesis of hepatitis C virus ushered in the era of direct acting antiviral agents. These agents have been the subject of intensive research in the last two decades, leading to the development of drug classes such as protease inhibitors (e.g., grazoprevir), NS5A inhibitors (e.g., daclatasvir) and NS5B inhibitors (e.g., sofosbuvir). While many are still under development, several have been approved for hepatitis C therapy. A number of studies investigating the combination of direct acting antiviral agents with or without pegylated interferon and/or ribavirin for the treatment of chronic hepatitis have demonstrated sustained virologic response of > 90%. Given the array of direct acting antiviral agents currently available, the present landscape of hepatitis C therapy is now characterized by a gradual transition to all-oral interferon-free regimens. Despite these milestones, the WHO global target of eliminating hepatitis C as a public health problem by 2030 seems uncertain. In this review, we provide a concise account of the evolution and advancements in the development of anti-HCV regimens.

**Key words:** hepatitis C; anti-HCV; antiviral; DAA; interferon; oligonucleotide.

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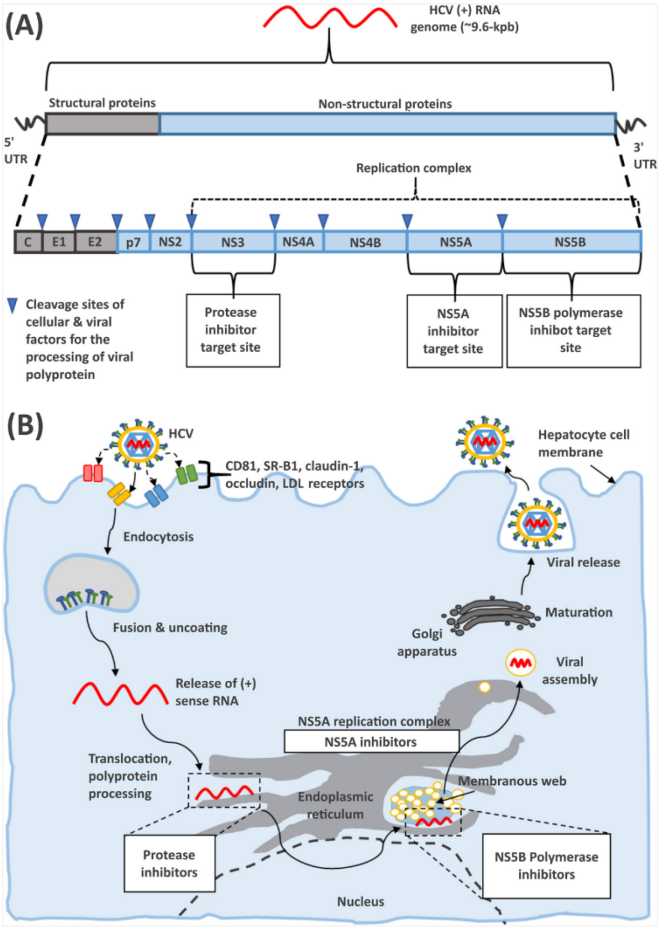
## Introduction

Since its discovery in 1989 [1], hepatitis C virus (HCV), one of five viruses most implicated in viral hepatitis, has been extensively studied. The virus belongs to the genus *Hepacivirus* of the *Flaviviridae* family. It is an approximately 9,600 bp positive sense single stranded ribonucleic acid (RNA) virus with a long open reading frame (bordered by 5' and 3' untranslated regions) that codes for structural (core, E1 and E2) and nonstructural (p7, NS2, NS3, NS4A, NS4B NS5A and NS5B) proteins [2] (Figure 1A). At least 6 genotypes and more than 80 subtypes with varying global and regional distributions have been identified [3,4]. Because HCV is bloodborne, it is predominantly transmitted via practices that result in exposure to infected blood. Upon transmission, HCV makes its way to the liver cells (hepatocytes) [5] and undergoes a replication cycle typical of a positive sense RNA virus, and ultimately produces ample viruses that infect other hepatocytes. Because the life cycle and pathogenesis of HCV has been well studied and depicted [2], an illustration to highlight the regions of the life cycle targeted by anti-HCV agents is presented (Figure 1B). If left untreated, HCV infection can result in complications such as scarring of the liver (cirrhosis), hepatocellular carcinoma (liver cancer), liver failure, and even death.

With no fewer than 58 million people estimated to be chronically infected by HCV globally [6], hepatitis is a serious cause for concern. Cirrhosis and hepatocellular carcinoma, which often result from chronic HCV infection, account for the majority of

recorded HCV mortalities [6]. Although hepatitis C is distributed globally, Europe and the Eastern Mediterranean Region have the highest burden of disease, with 12 million people chronically infected in each region [6]. People who inject drugs (PWID) and human immunodeficiency virus (HIV) positive men who have sex with men have been identified as major high-risk populations [7]. Unlike other hepatitis viruses, there is currently no licensed prophylactic or therapeutic vaccine against HCV infection. Hence, treatment and management of the disease largely relies on antiviral therapy. Owing to the diversity and large mutational profile of HCV, as well as the limited availability of pan-genotypic anti-HCV agents, identification of infecting genotype typically precedes treatment of HCV infection.

**Figure 1.** HCV and antiviral targets.




**A**) HCV genome and direct acting antiviral (DAA) target sites. The genome is approximately 9600 bp in length and is flanked by 5' and 3' untranslated regions (UTRs). Viral polyprotein is cleaved at different points, giving rise to 10 proteins (structural and non-structural). Inhibitors target NS3, NS5A and NS5B regions. **B**) Targets of DAAs in the life cycle of HCV. Following viral interaction with cell surface receptors and proteins, HCV enters the hepatocyte via a receptor mediated endocytosis. Direct cell-to-cell viral transmission can also occur. In the cell, RNA genome is released and translated in the rough endoplasmic reticulum. The translation process generates a single polyprotein of about 3000 amino acids long. Protease inhibitors target viral NS3 enzyme at this point. Replication of viral RNA occurs at the membranous web. The NS5A and NS5B inhibitors target and disrupt RNA replication and viral assembly process. HCV, hepatitis C virus. NS, non-structural; SR-B1, scavenger receptor class B type 1; LDLR, low density lipoprotein receptor.

## Pre-Direct acting antiviral era

The early years of hepatitis C therapy were marked by the use of interferon. Interferon alfa (IFN-α) modulates the functions of the immune system, triggering a general antiviral state in cells. Interestingly, its usage commenced even before HCV was fully identified. A study conducted in 1986 to investigate the effect of recombinant human IFN-α for the treatment of chronic non-A, non-B hepatitis showed that interferon could normalize serum aminotransferase levels and improve liver histology [8]. This foremost finding sparked, in large part, the race for anti-HCV therapy. Upon identification of HCV, it became possible to assess the success of the therapy through the long-lasting disappearance of HCV RNA from the serum, a yardstick commonly termed 'sustained virological response (SVR)'. With the standard interferon therapy, only about 15 to 20% of patients with chronic hepatitis C attained SVR [9]. Subsequent advances in interferon research led to the emergence of pegylated interferon (also known as peginterferon), a chemically modified form of the standard interferon. It is formulated with the inclusion of polyethylene glycol ('PEG') which helps to increase the half-life of the drug, thereby decreasing dosage frequency. This improved pharmacokinetic profile made it possible to administer Peg-IFN as a weekly dose [10]. Two prominent commercially available peginterferon (Peg-IFN) formulations are Peg-IFN alfa-2a and Peg-IFN alfa-2b. PEG-IFN alfa-2a is covalently attached to a 40 kDa branched chain polyethylene glycol moiety while PEG-IFN alfa-2b is bound to a single linear 12 kDa polyethylene glycol molecule [10]. Due to their different polyethylene glycol moieties, both Peg-IFNs have different pharmacokinetic profiles. Compared with standard IFN monotherapy, overall, SVR rates roughly doubled in Peg-IFN alfa-2a and Peg-IFN alfa-2b monotherapy clinical trials which were completed in the year 2000 [11].

In order to improve the hepatitis C treatment efficacy of IFN, ribavirin, a nucleoside analog with broad antiviral activity was used as a supplementary regimen [12]. Addition of ribavirin helps to hasten viral clearance early in the treatment course, thus, decreasing

the rate of relapse [13]. The drug is thought to function via direct (interference with RNA capping, polymerase inhibition, lethal mutagenesis) and indirect (immunomodulatory effects, inhibition of inosine monophosphate dehydrogenase) mechanisms [12]. It has also been suggested that the drug could increase the activity of IFN-sensitive target genes [14]. Like interferon, ribavirin had been available for medical use prior to the discovery of HCV [15]. It is often used in combination with another regimen since its usage as monotherapy is not effective for the treatment of chronic hepatitis C infection [16]. Many clinical trials that evaluated the combination of Peg-IFN-α and ribavirin for the treatment of hepatitis C infection in children, adolescents, and adults, demonstrate relatively high SVR rates. For example, 53% SVR was achieved in children treated with a combination of Peg-IFN-α-2a and ribavirin as compared with 21% SVR in those treated with Peg-IFN-α-2a and placebo [17]. In another study, pediatric patients were treated with a combination of Peg-IFN-α-2a and ribavirin. SVR was achieved in 57% of the patients infected with genotypes 1, 4, 5 and 6, and 94% in those infected with genotypes 2 and 3 [18].

Treatment of HCV infected patients with Peg-IFN and ribavirin were unfortunately associated with several undesirable and adverse effects that often led to premature withdrawals from therapy. Commonly reported side effects include headache, myalgia, rigors, pyrexia, arthralgia, nausea, loss of appetite, weight loss, alopecia, diarrhea, rash/dermatitis, injection site inflammation, pruritus, dyspnea, fatigue, insomnia, irritability and depression [10]. In clinical studies, serious side effects often warranted dose modifications which could lead to reduced response. Another drawback was the considerably long treatment durations, given that IFN was mainly administered by injection. Despite the standard 48 weeks of therapy, patients infected with genotype 1 often displayed suboptimal treatment outcomes during the pre-direct acting antiviral (pre-DAA) era. It was conjectured that better outcomes could be achieved if treatment duration was increased. Studies have investigated the safety and efficacy of PEG-IFN plus ribavirin for a period of 48 and 72 weeks. A multicenter randomized study showed

**Table 1.** Historical landmarks in the development and usage of HCV antivirals.

| Year | Major milestone |
|---|---|
| 1975 | Identification of non-A, non-B hepatitis |
| 1986 | Approval of interferon for medical use |
| 1986 | Proof of concept – using recombinant human alfa interferon for the treatment of chronic non-A, non-B hepatitis in patients |
| 1989 | Discovery of HCV |
| 1995 | High-throughput screening for NS3-NS4 serine protease inhibitors |
| 1996 | High-throughput screening for NS5B RNA-dependent RNA polymerase inhibitors |
| 1998 | Combination of ribavirin with IFN alfa improved SVR |
| 1999 | Structure-assisted polymerase inhibitor design |
| 2001 | Introduction of pegylated interferon alfa |
| 2001 | FDA approval of Peginterferon alfa-2b for the treatment of chronic hepatitis C |
| 2002 | Confirmation of NS3 protease as a therapeutic target and the commencement of clinical trial for the first HCV protease inhibitor, ciluprevir (BILN-2061) |
| 2004 | Proof of concept for the first nucleoside polymerase inhibitor, valopicitabine (NM283) |
| 2005 | Establishment of a high-resolution structure of NS5A domain I and structure-assisted design of NS5A inhibitor |
| 2006 | Proof of concept for the first non-nucleoside inhibitor (HCV-796) |
| 2006 | Proof of concept for non-immunosuppressive cyclophilin inhibitors, alisporivir |
| 2008 | Proof of concept for the first NS5A inhibitor, daclatasvir |
| 2008 | Commencement of the first interferon-free clinical trial (INFORM-1 study) |
| 2009 | Determination of HCV genotype in patients as an important pre-treatment requirement |
| 2010 | Commencement of clinical development of an entry inhibitor, ITX-5061, which targets SRB1 |
| 2010 | Discovery of PSI-7977 (sofosbuvir), an NS5B inhibitor |
| 2011 | Approval of the first class of DAAs (protease inhibitors; boceprevir and telaprevir) successfully used in the clinic |
| 2012 | Proof of concept for miR-122 antagonists (miravirsen) in patients |
| 2013 | Approval of the first ribonucleoside analog inhibitor, sofosbuvir, for the treatment of chronic hepatitis C |
| 2014 | Approval of second generation NS3/4A protease inhibitor, simeprevir, by the FDA |
| 2014 | Commencement of more established usage of interferon-free DAA based therapy for chronic HCV infection |
| 2015 | Inclusion of daclatasvir in WHO's list of essential medicines |
| 2016 | First report of DAA therapy in children and adolescents |
| 2016 | FDA approval of the first pan-genotypic fixed-dose combination tablet (consisting of sofosbuvir and velpatasvir) for the treatment of chronic hepatitis C infection by all six major HCV genotypes |
| 2013-2020 | Evaluation of all-oral DAA and other combination therapies, as well as advocacy for pan-genotypic regimens |

This table was compiled from the following references and resources: [1,2,34,50,56-67].

that relapse rate was significantly reduced following 72 weeks of therapy [19]. In another study, there was no significant difference in the treatment outcome between patients subjected to 48 and 72 weeks of PEG-IFN plus ribavirin therapy, although benefit from extended treatment duration was observed in patients with low-level viremia at week 12 [20]. Even though extension of treatment duration beyond 48 weeks was thought to be a beneficial strategy to address the challenge of unresponsive genotype 1 infection, extended treatment duration could substantially affect patients' adherence to therapy.

The potential of amantadine (an antiviral drug) to improve both biochemical and virological markers in patients with hepatitis C who were previous non-responders to interferon treatment was reported by Smith JP in 1997 [21]. The effect of amantadine in combination with IFN and/or ribavirin was evaluated in subsequent studies. Among the early studies, promising SVR rate was reported in a study where triple (INF/ribavirin/amantadine) therapy was used for one year in patients with interferon-nonresponsive chronic hepatitis C [22]. The report enlivened hopes of improving the success of therapy in patients infected by HCV genotype 1. However, other studies did not find significant benefit when amantadine was used in combination with IFN and ribavirin [23]. These problems underscored the need for alternative hepatitis C regimens.

## The Direct acting antiviral (DAA) era

Through the years, improved understanding of HCV pathogenesis spurred further breakthroughs in anti-HCV research (Table 1), resulting in the development of more tolerable oral agents known as direct acting antivirals (DAAs).

Unlike interferon and ribavirin, DAAs are more specific in action and have since been used as mono- or combination therapies with or without interferon and ribavirin. Most patient populations treated with DAAs have attained cure rates of more than 90% [24]. Many of the present-day DAAs are inhibitors designed to interfere with the intracellular replicative stage of HCV's life cycle, with principal viral targets being NS3/4A protease, NS5A protein, and NS5B RNA-dependent RNA polymerase (Figure 1B). Interestingly, over forty anti-HCV DAAs have attained phase two clinical trial status in the past two decades (Table 2).

*Protease inhibitors*

The nonstructural protein 2 (NS2) and NS3 of HCV play crucial roles in replication of the virus. NS2 functions as a cysteine protease and also acts as a cofactor in the assembly of the viral components [25,26]. Its protease activity splits NS2 and NS3 from the growing polyprotein during translation, making them freely available to aid the RNA replication process [25,26]. NS3 on the other hand is an enzyme that has helicase activity (C-terminal region), and at the same time, functions as a serine protease (N-terminal region) [27]. With the help of its membrane-bound cofactor (NS4A), the NS3 enzyme cleaves the HCV polyprotein to release five nonstructural proteins (NS3, NS4A, NS4B, NS5A, and NS5B) necessary for replication [28] (Figure 1). Unlike NS2, which was met with challenges in the development of a potent inhibitor [2], insights into the crystal structure of the NS3 protease and the availability of biochemical assays for investigation propelled advances in NS3/4A serine protease inhibitors (Table 2) which are now either being used or under evaluation for targeted treatment of HCV infection.

In addition to the ones already approved, several serine protease inhibitors are currently under development. Structurally, these protease inhibitors are mainly linear (e.g., telaprevir, boceprevir) or macrocyclic (e.g., asunaprevir, simeprevir, vaniprevir) chemical compounds. The possible mechanism of NS3/NS4A serine protease inhibitors include: (i) interference with NS4A-aided activation of the enzyme (ii) binding to the structural zinc site and (iii) binding to the active site [27].

*NS5A inhibitors*

The HCV's NS5A is a proline-rich phosphoprotein with an ability to bind to zinc [29]. It is an important constituent of the HCV replication cycle, as it interacts with host cell proteins (e.g., kinases) as well as important viral products (e.g., core and RNA) to regulate viral replication and assembly [30]. NS5A inhibitors are designed to disrupt RNA replication and viral assembly process. For example, daclatasvir, the first NS5A inhibitor, prevents replication and assembly by binding to NS5A, targeting its cis- and trans-acting functions with a resultant disruption of the replication complexes [31]. It has also been shown that the inhibitor can hinder intracellular viral RNA synthesis as well as the assembly/secretion of the viral particle [32].

*NS5B inhibitors*

NS5B is an RNA-dependent RNA polymerase essential for the replication of HCV. The enzyme is a 66 kDa protein with a characteristic finger-palm-thumb structural motif typical of many viral polymerases [33].

**Table 2.** Compendium of notable antiviral agents against hepatis C virus.

| Antiviral agents | Phase | Status/Year of approval | Comment |
|---|---|---|---|
| **Non-direct acting antivirals** | | | |
| Ribavirin | approved | 1986 | agent with a broad-spectrum activity against many DNA and RNA viruses including HCV |
| Peginterferon alfa-2b | FDA approved | 2001 | a form of recombinant interferon used as part of combination therapy to treat chronic Hepatitis C |
| Peginterferon alfa-2a | FDA approved | 2002 | a form of recombinant interferon used as part of combination therapy to treat chronic Hepatitis C |
| **DAAs - Protease inhibitors** | | | |
| Boceprevir | FDA approved | 2011 | no longer widely used |
| Telaprevir | FDA approved | 2011 | withdrawn/discontinued due to waning demand |
| Paritaprevir | approved | 2014 | – |
| Simeprevir | FDA approved | 2014 | second generation protease inhibitor<br>excluded from the WHO's list of essential medicines in 2019 |
| Asunaprevir [BMS-650032] | approved | 2016 | withdrawn in 2017 due to rapidly evolving HCV treatment landscape |
| Grazoprevir | FDA approved | 2016 | second generation protease inhibitor |
| Glecaprevir | FDA approved | 2017 | – |
| Voxilaprevir | FDA approved | 2017 | – |
| Vaniprevir | approved | – | investigational<br>approved for hepatitis C treatment in Japan |
| Danoprevir | phase 3 | under investigation | investigational |
| Faldaprevir | phase 3 | discontinued | investigational |
| Narlaprevir | phase 3 | under investigation | investigational |
| Ciluprevir [BILN-2061] | phase 2 | discontinued | first protease inhibitor to enter clinical development<br>development was discontinued due to toxicity in animals |
| GS-9256 | phase 2 | – | demonstrates potent activity against HCV genotype 1 |
| Sovaprevir | phase 2 | – | investigational |
| Vedroprevir | phase 2 | – | investigational |
| Deldeprevir (Neceprevir) | - | – | investigational |
| **DAAs - NS5A inhibitors** | | | |
| Ledipasvir | FDA approved | 2014 | – |
| Ombitasvir | FDA approved | 2014 | – |
| Daclatasvir | FDA approved | 2015 | – |
| Velpatasvir | approved | 2016 | – |
| Elbasvir | FDA approved | 2016 | – |
| Pibrentasvir | FDA approved | 2017 | – |
| Ravidasvir | phase 3 | under investigation | investigational |
| GSK2336805 | phase 2 | – | investigational |
| Odalasvir | phase 2 | under investigation | investigational |
| Ruzasvir | phase 2 | under investigation | pan-genotype NS5A inhibitor |
| Samatasvir | phase 2 | under investigation | investigational |
| **DAAs - NS5B inhibitors** | | | |
| Dasabuvir | approved | 2014 | non-nucleoside inhibitor |
| Sofosbuvir | FDA approved | 2014 | prodrug; nucleotide analog |
| Beclabuvir | phase 3 | under investigation | investigational |
| Deleobuvir | phase 3 | – | non-nucleoside inhibitor |
| ABT-072 | phase 2 | under investigation | non-nucleoside inhibitor |
| Filibuvir | phase 2 | discontinued | non-nucleoside inhibitor |
| IDX184 | phase 2 | discontinued | – |
| Lomibuvir | phase 2 | – | non-nucleoside inhibitor |
| Mericitabine | phase 2 | under investigation | nucleoside analog inhibitor |
| Radalbuvir | phase 2 | under investigation | investigational |
| Setrobuvir | phase 2 | discontinued | – |
| Tegobuvir | phase 2 | discontinued | – |
| TMC647055 | phase 2 | under investigation | – |
| Valopicitabine | phase 2 | discontinued | prodrug of the nucleoside analog 2'-C-methylcytidine |
| VCH-759 | phase 2 | – | – |
| VX-135 | phase 2 | – | – |
| **Other agents** | | | |
| Alisporivir | phase 3 | under investigation | cyclophilin inhibitor |
| Miravirsen | phase 2 | under investigation | microRNA-122 inhibitor |
| NIM811 | phase 2 | – | cyclophilin inhibitor |
| SCY-635 | phase 2 | under investigation | cyclosporine-based analog |
| VX-135 | phase 2 | under investigation | uridine nucleotide analog prodrug |

This table was compiled from the following references and resources: [3,15,37,65,67-70]. There are several anti-HCV agents under development. In this table, effort was made to include notable agents that have entered at least a phase two trial owing to their imminent clinical significance. Any selection bias is unintended. DAAs, Direct acting antivirals.

Using the RNA genome of HCV as a template, NS5B can directly synthesize RNA without the need for a primer [34]. However, its efficiency, particularly in accommodating newly synthesized RNA, is subject to the ability of its fist-like structure to undergo extensive rearrangements and conformational changes to permit binding with RNA and incoming nucleotides [35]. This pivotal role of NS5B in viral RNA synthesis has been greatly harnessed in the quest for potent HCV antiviral agents. The identification of well conserved active-site residues in NS5B [35] also enhanced the development of several nucleotide and nucleoside inhibitors (Table 2) that target the active site of the enzyme, thereby terminating the growing RNA chain [36]. In addition, the allosteric sites on NS5B have also been targeted to develop non-nucleoside inhibitors such as beclabuvir and benzimidazole (thumb site I inhibitors), GS-9669 (thumb site II inhibitor), and dasabuvir (ABT-333) (palm site inhibitor) [34]. However, non-nucleoside inhibitors do not exhibit broad genotype activities [34], and their barrier to resistance is significantly lower than nucleoside inhibitors [37].

## Anti-HCV agents and treatment patterns

The gradual but relatively steady progress in the development of anti-HCV regimens (Table 1 and 2) is a harbinger of an imminent triumph in the fight against HCV infection. Many of the available anti-HCV agents are now employed as standard hepatitis C regimens for treatment-naive and treatment-experienced patients using different drug combinations and treatment considerations.

*Single drug usage*

Monotherapy for hepatitis C using an agent or drug with a single formulation is uncommon. Many treatment regimens involve combination therapy of more than one antiviral agent, or the use of a single formulation with components from multiple antiviral agents. Several attempts made in the past to investigate the efficacy of monotherapy for hepatitis C left much to be desired. For example, results from several clinical trials evaluating ribavirin monotherapy for the treatment of chronic hepatitis have been shown to disfavor its use as a mono-therapeutic regimen [38].

*Dual drug combination*

The combination of two different antiviral agents (e.g., simeprevir and sofosbuvir) or the use of an agent formulated with the components of two different antiviral agents (e.g., elbasvir-grazoprevir) for the treatment of hepatitis C gained prominence over the years. As compared with monotherapy, use of dual combination therapy improved patients' outcomes for the treatment of hepatitis C [39]. While some agents such as glecaprevir-pibrentasvir, elbasvir-grazoprevir, daclatasvir in combination with sofosbuvir, simeprevir in combination with sofosbuvir, etc., have been approved for the treatment of chronic hepatitis C, other dual drug regimens are still undergoing clinical trials with some promising results.

*Regimens with more than two agents*

Given that combination regimens yield propitious results, efforts have been intensified to identify regimens with high efficacy, broader HCV genotype coverage, and shorter treatment durations. Combination of agents from different classes of antiviral agents have thus been tried. Successes in clinical trials involving triple regimens such as the combination of sofosbuvir (a nucleotide analog NS5B polymerase inhibitor), velpatasvir (an NS5A replication complex inhibitor), and GS-9857 (an NS3/4A protease inhibitor) [40] or sofosbuvir, velpatasvir, and voxilaprevir (a protease inhibitor) [41], among other trials, are no doubt instrumental to the approval of the first pan-genotypic fixed dose tablet, sofosbuvir-velpatasvir-voxilaprevir. Treatment regimens involving more than three agents also abound, especially for the treatment of difficult-to-cure hepatitis C patients. This was to improve efficacy with lowered chances of resistance selection. An example is the open-label phase 2 study that evaluated the efficacy of daclatasvir-based quadruple therapy (involving a combination of daclatasvir, asunaprevir, pegylated interferon, and ribavirin) in sixty patients who were null or partial responders to previous hepatitis C treatments. The quadruple regimen yielded SVR as high as 95% at 12 weeks of treatment [42]. Many all-oral regimens used as combination therapies have also demonstrated satisfactory SVR rates (Table 3).

*Therapy based on infecting genotype*

Targeted therapy was the bane of DAAs as their development evolved, probably because of the differences in genotypes and variations in their distributions. Although it has been shown that genotypes 1, 2 and 3 appeared to be distributed globally, the so called 'endemic' strains of genotype 1 and 2 are common in West Africa while genotype 3 is frequently seen in South Asia [43]. Meanwhile, the Middle East and Central Africa, Southern Africa, and Southeast Asia have a predominance of genotypes 4, 5 and 6, respectively [43]. In addition, relative to other genotypes, infection with HCV genotype 3 was in the

past believed to be difficult to treat and considered the most treatment-refractory of all genotypes in the era of DAA. Some of the currently available regimens are designed for specific genotypes. An example is elbasvir-grazoprevir (Zepatier) for treatment-naive and treatment-experienced patients infected by genotype 1 or 4. However, approval of the first pan-genotypic fixed-dose combination tablet in 2016 (Table 1) further invigorated efforts towards the development of more pan-genotypic regimens. It is expected that the availability of effective pan-genotypic regimens would reduce and ultimately eliminate the usual need to ascertain the infecting HCV genotype prior to administering treatment, thus, reducing the cost of managing hepatitis C. With the current urge for pan-genotypic regimen, it can be envisaged that the treatment of hepatitis C infection based on infecting genotype would become unpopular in the next few years.

*Special HCV infected population*

Certain HCV infected populations such as children, pregnant women, people who inject drugs (PWID), men who have sex with men (MSM), kidney transplant patients, patients with HIV coinfection, and patients with renal impairment need special considerations for HCV antiviral therapy. For example, non-hepatic organ dysfunction, liver-related morbidity and mortality, and overall mortality are thought to be higher in HCV patients coinfected with HIV compared with HCV-monoinfected patients [44]. In the same vein, PWID and MSM may have a high burden of chronic HCV infection. Also, many clinical trials of established anti-HCV drugs, or agents under development, are conducted using adult subjects, leaving children with fewer options of potent therapies. To ensure initiation of treatment at the most appropriate time and the administration of the right regimens for better clinical outcomes, treatment of these unique populations with the currently available HCV antiviral regimens are initiated using expert recommendations and consensus guidelines. This is however not the focus of this article. More updated information on recommended drug combinations and advice for the treatment and management of these populations are available online [45].

## Other antiviral agents

Beside the DAAs, there are other antiviral substances that have either been or are still being tested for potential anti-HCV effects. Some notable agents including C5A and small-molecule host-targeting agents (e.g., microRNA-122 [miR-122] antagonists,

**Table 3.** Clinical evaluation reports of some all-oral DAA combinations for the treatment of HCV infection.

| DAA Regimen | Targeted HCV genotype | Participants (N) | Population | Treatment Duration (weeks) | SVR post-treatment (week) | Overall SVR | Ref |
|---|---|---|---|---|---|---|---|
| (SOF + DCV ± RBV), (SOF + DCV/SMV ± RBV), (SOF + RBV), and (SOF + DCV ± RBV) | 1, 2, 3 | 527 | Cirrhotic and non-cirrhotic adult patients with genotype 1, 2 or 3 infection | 12 and 24 | 12 | > 90% | [71] |
| SOF–VEL | 1,2,4,5, 6 | 624 | Cirrhotic and non-cirrhotic adult patients who had chronic infection with HCV genotype 1, 2, 4, 5, or 6 | 12 | 12 | 99% | [72] |
| GZP–EBV | 1, 4, 6 | 316 | Cirrhotic and noncirrhotic treatment-naive adults with genotype 1, 4, or 6 infection | 12 | 12 | 95% | [73] |
| (ABT-450/r–OBV + DAV + RBV) | 1 | 724 | Adult patients with genotype 1a or 1b chronic HCV infection | 12 | 12 | > 90% | [74] |
| OBV + PTV-r ± RBV | 4 | 467 | Non-cirrhotic chronic HCV genotype 4 infected adults | 12 | 12 | > 90% | [75] |
| LDV–SOF | 4 | 20 | Cirrhotic and noncirrhotic adult patients with chronic HCV genotype 4 infection | 12 | 12 | 100% | [76] |
| LDV–SOF | 4 | 44 | Treatment-naïve treatment-experienced patients with HCV genotype 4 infection | 12 | 12 | 93% | [77] |
| SMV + SOF | 1 | 310 | Non-cirrhotic treatment-naïve and treatment-experienced chronic HCV genotype 1a or 1b infected patients. | 8 and 12 | 12 | 83% (8 wks) 97% (12 wks) | [78] |
| DCV + SOF ± RBV | 1, 2, 3 | 211 | Adult patients with chronic HCV genotype 1, 2, or 3 infection | 12 and 24 | 12 | > 88% | [79] |
| DCV + SOF + RBV | 3 | 50 | Adult patients with chronic HCV genotype 3 infection with advanced fibrosis or compensated cirrhosis | 12 and 16 | 12 | 90% | [80] |

SOF: sofosbuvir; DCV: daclatasvir; SMV: simeprevir; RBV: ribavirin; GZP: grazoprevir; EBV: elbasvir; OBV: ombitasvir; ABT-450/r: ABT-450/ritonavir; DAV: dasabuvir; LDV: ledipasvir; VEL: velpatasvir; PTV–r: paritaprevir–ritonavir; OBV: ombitasvir; DAA: Direct acting antiviral.

cyclophilin inhibitors, viral entry inhibitors) are briefly described here.

C5A is an amphipathic α-helical peptide derived from the membrane anchor domain of the NS5A protein of HCV. By inactivating both intra- and extracellular infectious particles, C5A is able to prevent de novo HCV infection and suppress ongoing ones. The peptide has been shown to elicit minimal toxicity in vitro [46]. ITS-5061, a viral entry inhibitor which has attained a phase 2 clinical evaluation [2], is a host-targeting agent (HTA) that inhibits both the uptake of high-density lipoprotein through the scavenger receptor class B type I (SR-BI) receptor (Figure 1) and the uptake of the HCV particle [47]. Miravirsen is another HTA. It is a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide that inhibits the function of mature miR-122 by sequestering it in a highly stable heteroduplex. This agent established a long-lasting suppression of HCV viremia without evidence of side effects or viral resistance in chronically HCV-infected non-human primates [48]. Similarly, in a phase 2 study of patients infected with HCV genotype 1, the use of miravirsen favored a prolonged dose-dependent reduction in the levels of HCV RNA without evidence of viral resistance [49]. Alisporivir (DEBIO-025), NIM811, and SCY-635 are prominent cyclophilin inhibitors that have attained at least a phase 2 clinical evaluation (Table 2). Although Alisporivir is recognized for its pan-genotypic effects, cases of pancreatitis were observed when combined with Peg-IFN-α. However, this agent shows promise in the current drive for IFN-free regimens.

Other nucleic acid-based agents such as aptamers, small interfering ribonucleic acids (siRNAs), ribozymes, and antisense oligonucleotides targeting HCV genome have also been proposed and are being investigated for their potentials as alternative HCV regimens.

## Challenges in HCV antiviral development and therapy

*Antiviral resistance and DAA Treatment failures*

Antiviral resistance is probably the greatest hurdle in the development of efficacious hepatitis C treatment regimens. Owing to its highly error-prone RNA-dependent RNA polymerase and large viral populations, HCV exhibits a profound genetic diversity with varied mutational profiles. The high mutability and emergence of multiple quasispecies during replication gives rise to many resistance-associated variants (RAVs) that harbor amino acid substitutions capable of conferring resistance to one or more DAAs [3]. These amino acid substitutions, often termed resistance-associated substitutions (RAS), could be present before (baseline), during, or after treatment (treatment-emergent) with anti-HCV agents. Agents such as NS5B nucleotide/nucleoside analogs designed to target conserved active sites demonstrate higher barriers to resistance than their counterparts such as NS5A inhibitors or protease inhibitors which are often characterized with low barriers to resistance. These variations in HCV genotypes, subtypes and quasispecies have impacted antiviral development over the years. During the pre-DAA era when interferon was largely used, genotype 1 was the most difficult to treat. However, genotype 3 poses the greatest challenge in the current combination DAA era partly because much of the earlier efforts were directed to remedy the hard-to-treat yet widely distributed genotype 1 [50]. However, other patients' baseline characteristics such as previous treatments or presence of cirrhosis play a role in these resistance mutations [50].

Despite the proven efficacy and tolerability of the newer anti-HCV regimens, treatment failures are often encountered, and factors responsible remain to be fully understood. Many of these failures are however attributed to treatment-emergent RAS. For example, in HCV genotype 1a infected patients that failed therapy with daclatasvir or ledipasvir, Q30H/R, Y93H and L31M are the most prevalent treatment emergent NS5A RAS, meanwhile L31M and Y93H are the most common in patients infected by HCV genotype 1b [51]. Similarly, the most common emergent RAS following daclatasvir treatment failure in patients infected by genotype 3 is Y93H [51]. Myriad treatment-emergent RAS have since been reported [51] and explicitly documented (reviewed in [3]). Treatment failures may require patients to be placed on a different class of anti-HCV agent.

*Cost of anti-HCV drug development and therapy*

Other notable challenges involve concerns surrounding the expensive nature of HCV therapy. The newer and more tolerable anti-HCV drugs that are expected to proffer better alternatives to the older interferon regimens are extremely costly. For instance, previous reports indicate that a single pill of sofosbuvir (Sovaldi) costs approximately $1,000, and approximately $84,000 is required for a twelve-week treatment course [52]. Similarly, simiprevir (Olysio), which is of a six-to-twelve-month treatment duration, is estimated to cost around $23,600 per month [52]. These high costs are related to the developmental rigors of successfully introducing a potent drug into the market;

some drug candidates that show great promise following preclinical studies fail in early clinical trials, while some others fail at later phases of more robust clinical trials for reasons such as toxicity or severe adverse effects. In fact, some drugs are withdrawn from the market several years after approval. For example, owing to the availability of more superior agents, the manufacture of boceprevir (Victrelis), a first-generation protease inhibitor used to treat HCV genotype 1, has been discontinued in the United States. These massive losses incurred from failed drugs are also factored in by the pharmaceutical companies when determining the price for successful drugs. These huge costs however make the drugs unavailable to those who need it the most, especially in low resource areas. However, good progress has been made in the pricing of medicines. Despite high prices, many high-income countries (e.g., Australia, Scotland, and France) disclosed decisions to make treatment available for all persons infected with HCV, with minimal co-payments [53]. Middle income countries that intend to screen and treat a large percentage of their populations have so far been able to negotiate considerable price reductions. Through voluntary licensing agreements, low-income countries can also benefit from generic versions of new HCV drugs which can be offered for under $500 per patient [53]. A study estimated that a combination of two DAAs (generic sofosbuvir-daclatasvir combination regimen) could be produced for as low as $200 per patient for a 12-week treatment course [54]. Although major progress in the pricing of hepatitis C medicines has been made, the willingness of the governments across the continents to explore the available options and prioritize testing and treatment is pertinent to eradicating the disease.

*Eliminating Hepatitis C by 2030*

In 2014, the World Health Assembly requested the World Health Organization (WHO) to assess the feasibility of, and the approaches necessary for the elimination of hepatitis B and C. The move gave rise to the WHO's global target of eliminating the diseases as public health problem by 2030 [53]. It was hoped that prevention strategies and adequate screening and treatment would help achieve this goal. Considering some of the highlighted issues surrounding hepatitis C therapy, the hope of attaining this 2030 target is wrought with challenges. Moreover, a study that was presented at the 2019 International Liver Congress of the European Association for the Study of the Liver (EASL) showed that, of the 45 high-income countries and territories, only nine (the United Kingdom, Switzerland, Spain, South Korea, Japan, Italy, Iceland, France, and Australia) were estimated to be on track towards eliminating HCV by 2030 [55]. Three (Malta, Germany, and Austria) were projected to eliminate HCV by 2040, and another three (Saudi Arabia, Ireland, and the Netherlands) by 2050. Surprisingly, projections from the study also revealed that thirty high-income countries would not eliminate HCV before 2050 [55]. What then is the fate of low- and middle-income countries? Similar to the WHO, World Hepatitis Alliance, and other organizations continue to work with the governments of various countries, only time will tell if hepatitis C infection can become a historical footnote by the end of this decade.

## Conclusions and future directions

Given the absence of a licensed HCV vaccine, the use of anti-HCV agents and drugs has been the mainstay of hepatitis C therapy for many years now. Massive efforts have been and are still being made towards discovering the most ideal treatment regimen for hepatitis C infection. Thus far, the emergence of DAAs has greatly revolutionized the terrain of HCV therapeutics; treatment durations have become shorter (8-12 weeks), drugs are more tolerable with minimal side effects, and SVR rates have increased to over 90% in most chronically infected HCV populations. However, more needs to be done to bolster these excellent advancements in order to reduce and ultimately eliminate HCV infection as a public health threat. First, future studies should pay attention to those with previous treatment failures. Although impressive cure rates have been recorded so far, it is pertinent to unravel the precise cause of treatment failures that are sometimes observed in a handful of hepatitis C patients enrolled for clinical trials of DAAs as well as those who had the usual treatments. Second, even though DAAs are considered more tolerable, it is important to properly evaluate their toxicity, particularly in vulnerable populations such as transplantation patients. Third, as the pursuit for better regimens and the need to circumvent viral resistance continues, further exploration of other promising options such as virucidal peptides derived from HCV NS5A [46], oligonucleotide-based therapy, small-molecule host-targeting agents (e.g., miR-122 antagonist, cyclophilin inhibitors, viral entry inhibitors, etc.), among others, might be worthwhile. Finally, accessibility to the currently approved anti-HCV agents should be prioritized. Although efficacious, these modern anti-HCV drugs are extremely expensive. Government

interventions are needed, especially in more endemic regions, to ensure global access to HCV treatment.

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## Authors' Contributions

Conceptualization: A.A.I and R.H.S.; writing-original draft preparation: A.A.I; writing-preparation of tables and figures, A.A.I, E.N.S.E.A.R., N.A.Z.M.A.; writing-review and editing: A.A.I, E.N.S.E.A.R., N.A.Z.M.A, N.M. and R.H.S.; funding acquisition: R.H.S. All authors have read and agreed to the published version of the manuscript.

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