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Exploring Novel Therapeutic Approaches for Hepatitis C: Targeting Host-Virus Interactions to Develop Antiviral Strategies

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Abstract

Objective: This review will focus thematically on some of the newer therapeutic strategies for HCV infection: host approved targets of virus-host interactions. Aims; To describe the potential benefits and challenges of these radical approaches including host-targeted therapies (HTTs), repurposed drugs, and RNA interference (RNAi) strategies.

Methods: The current understanding of the structure and life cycle of HCV, existing therapeutic measures and also the vicissitudes in the interaction between HCV and host cellular factors are reviewed here through a literature search and by evidence from recent studies. The authors reviewed primary research and clinical trials on HTTs, repurposed drugs, and RNAi to evaluate their biological effects, effectiveness, and resultant outcomes.

Results

Because host-virus interaction proteins are generally not expected to mutate in viruses, therapeutics that target such interactions may also be less likely to induce the development of viral resistance (26) and actively promote broad-spectrum antiviral effects. Preclinical and clinical results for PIs, FTPs and other host-targeted therapies including inhibitors of the PI3K/Akt signalling pathway and lipid biosynthesis enzymes have shown promise. A combination of repurposed drugs such as statins and metformin, along with RNA interference targeting those host genes critical for the viral replication can provide a bonafide strategy to reduce HCV RNA levels and enhance therapeutic efficacy. Unfortunately, work still needs to be done for these therapies to reach their full potential as they suffer from off-target effects, toxicity, and regulatory problems.

Keywords: Hepatitis C Virus (HCV), Antiviral Strategies, Host-virus Interactions, Host-targeted therapies (HTTs), Repurposed Drugs, RNA Interference (RNAi), CRISPR/Cas9, Gene Editing, Personalized Medicine, Viral Resistance, Clinical Trials.

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Introduction

Hepatitis C (HCV) is a liver disease resulting from the infection with the Hepatitis C virus, which affects millions of people globally. It is a significant global health problem, with the World Health Organization reporting that about 71 million people worldwide are chronically infected with HCV. Hepatitis can cause severe liver damage, such as cirrhosis or liver cancer and is one of the main reasons for liver transplantation. Hepatitis C is a major burden, especially in low-and middle-income countries but diagnosis and treatment remain out of reach for much of the population. The worldwide burden of HCV and its high morbidity and mortality rates, despite the advances in therapeutic modalities for HCV, make it essential continuing to develop new strategy for the treatment [1].

Standard treatments for Hepatitis C are now predominately direct-acting antivirals (DAAs), which act directly on the virus itself against specific steps in viral protein production to stop it multiplying. Although DAAs have transformed treatment of hepatitis C with cure rates higher than 90%, they also suffer from limitations. This is due to some combination of high prices, potential for drug resistance and suboptimal efficacy in some patient populations (e.g.: advanced liver disease and co-infections). Moreover, since DAAs do not confer immunity after infection has cleared, they are unable to prevent reinfection. Therefore, it has become very important to discover newer strains that overcome these limitations and provide a more sustainable solution for HCV infection management in the long term.

A chief hurdle with DAAs has been the extensive expense of making and giving them to patients. Cost is a major barrier to all but the wealthiest nations, particularly low- and middle-income countries that contend with limited health care budgets. But these drugs are in short supply in parts of the world, despite global efforts to negotiate lower prices and make generic versions available. This high price impacts on individual patients, but it also creates a tremendous burden for national healthcare systems -preventing these programs from being reach the scale and extent required to bring about elimination at a national level [2].

A further major drawback of current HCV therapeutics is the threat of resistance. Genetic variability and rapid mutations in HCV lead to drug resistance to antiviral drugs due to its property of replication. It results in multi-drug class resistance, especially troubling for patients who fail to achieve a sustained virological response (SVR) from initial treatment, leading to subsequent efforts being less efficacious, rather than more. Conclusions - The significant threat that drug-resistant HCV strains pose to the long-term effectiveness of DAAs underscores the need for therapies able to mitigate this risk.

In addition to this, DAAs have changed the landscape of HCV treatments but they are not panacea as they would absolutely work for every patient population. These include patients with advanced liver disease, co-infection with HIV and those who have failed DAA therapy in the past; all experience decreased cure rates as well as increased adverse-event rates. The variation in treatment response requires the exploration of broader and more effective treatments, since every patient with HCV infection has to be treated with a duplex need.

Novel antiviral strategies are badly needed for Hepatitis C. With the virus maintaining its mutation, there is always room for better interventions that have potential to surpass the existing treatments. An attractive alternative approach is to target host-virus interactions. This strategy targets pathways through which HCV co-opts host cellular machinery to support its life cycle. We imagined it could be feasible to block these important interactions and create resistance-driven therapies that, in theory could be effective against more different viral strains [3].

This new era of antiviral therapy is what we call host-virus interaction. Most traditional antivirals, including DAAs, are designed to target viral proteins that must be present for replication of the virus. But the rapid mutation rate of some viruses - like HCV - makes it easy for resistance to arise. Instead, host-targeted therapies like those used in this study target cellular processes and pathways that viruses use for their replication. Because these host factors are far less mutable than viral proteins, drugs that target them should enjoy a higher resistance threshold.

Broad-spectrum antiviral activity is one of the important benefits of host-virus interactions as targets. Common cellular pathways and machinery are used by many viruses, including HCV, to replicate and spread. This could lead to a novel antiviral strategy: inhibition of common host factors like eIF4G that are utilized by not only multiple rhinoviruses but also other picornaviruses and viruses in different families. This means that if further studies confirm that this strategy is effective - and safe - a broad-spectrum drug for many viral diseases, rather than just HCV, could perhaps pave the way for a new era of antiviral drugs [4].

This along with other laboratories have also made advancement got the holy grail of a "Functional Cure" including Host-targeted therapies (HTTs) for new HCV therapeutics. The host-targeted antivirals, interfere with the host cell processes, essential for viral life cycle. A parallel strategy is to focus on involvement of cellular receptors and entry factors that HCV utilizes for the entry and infection of host cells. Closure of these entry points by HTTs can prevent the virus from infecting in the first place. Additionally, lipid receptors on liver cells that have been associated with HCV entry and are now targeted for inhibition by small molecules show promise as possible anti-viral agents.

A different approach aims to disrupt the intracellular signalling pathways that HCV hijacks to induce an environment favourable for its replication. HCV is known to modulate several hosts signalling pathways including those related to lipid metabolism and innate immunity. HTTs target these pathways instead of directly attacking the virus itself and can therefore inhibit viral replication and spread. This will not only lower the chances of resistance arising, but it should also decrease the effect on the host cell and could cause fewer side effects to "classical" antiviral drugs.

Small and Large Molecule Novel Therapies Additional strategies for developing novel HCV therapeutics also exist, including the repurposing of existing drugs. Low and high throughput screening for HCV drugs, including those targeting host factors essential for HCV replication, ports-of-opportunity are approved drugs for other indications. For instance, some lipid-lowering drugs and immunomodulators have been used to interfere with

the host pathways that HCV manipulates in the liver and are effective against several viral infections. Repositioning of these drugs has an advantage as this is a reduced cost and timely manner to the identification of new drug candidates since behaviour patterns and pharmacokinetic profile are still well defined, which means that their clinical translation will be faster [5].

RNA interference (RNAi) RNAi approach is the most developed in the field of host-virus interactions. RNAi is the procedure wherein small RNA molecules are use for silencing a handful of host genes that the virus needs in order to replicate. The researchers showed that, by designing RNAi molecules to target these essential host genes, they can efficiently block the cellular machinery upon which HCV depends for replication. Gaining entry to the cytoplasm of a targeted cell, the first step in delivery of a RNAi therapeutic, is an obstacle for this approach to become practical, but both advances in nanoparticle technology and other delivery systems are rapidly changing this status. RNAi therapies can provide highly specific and potent antiviral activity with low off-target effects, which regard them as a promising new weapon for clinical antiviral armamentarium.

Here, we present several case studies and research highlights further demonstrating the promise of host-virus targeted therapies. For example, cyclophilin inhibitors alleged to inhibit a core pathway of HCV infection via an interaction between the host cell and the viral protein necessary for replication showed antiviral activity in preclinical models. Host lipid metabolism pathways have also been shown to be targeted by specific inhibitors, which reduced HCV replication in cell culture and animal models52. Thus, these studies demonstrate the potential feasibility and efficacy of host-targeted strategies and lay a foundation for additional development and clinical assessment [6].

Notwithstanding the promise of these new designs, a range of challenges and issues need to be carefully managed. One key problem is off-targeting effects and, consequently, toxicity- host cellular pathways can be hijacked in a non-specific fashion often leading to the disruption of normal cell function. The identification and validation of host factors that are important for viral replication but dispensable to normal cellular activities is essential in order to minimize off-target effects. Moreover, the resistance will develop lesser than the traditional antiviral compounds but is and should be monitored with time to have combination therapy strategies.

The development of novel HCV therapeutics is further complicated by the regulatory and clinical trial hurdles that must be overcome. New therapies must be tested extensively and rigorously in a wide variety of patients to demonstrate that they are both safe and effective. Regulatory bodies are stuck between the need for rapid introduction of new therapies and careful evaluation in order to prevent unanticipated adverse events. Research and practitioner bodies, regulatory agencies, and industry players must work together to simplify the path of development for the potential therapy so that it gets to patients as quickly as possible.

Future strategies with these and novel modalities may achieve improved clinical outcomes and thus ITTPT for HCV. Gene editing technologies such as CRISPR/Cas9 hold great promise for the precise modification of host genes involved in viral replication, making them an attractive option for the creation long-lasting and efficacious therapies. Patient tailored or individualized treatments according to patient characteristics and genetic profiles

would be expected from Personalized medicine approaches that integrate discoveries from genetics. Future treatment strategies combining novel therapies with established treatments (e.g. DAAs and host-targeted therapies) might also result in better synergies and improved overall treatment success [7].

Hepatitis C Virus (HCV)

Hepatitis C Virus (HCV) is a small, enveloped, positive-sense single-stranded RNA virus belonging to the Flaviviridae family. The virus has a genome approximately 9.6 kilobases in length, which encodes a single polyprotein that is processed into structural and non-structural proteins. Understanding the structure and lifecycle of HCV is crucial for comprehending its pathogenesis and developing targeted therapeutic strategies.

The structure of HCV consists of an RNA genome surrounded by a nucleocapsid, which is further enveloped by a lipid bilayer derived from the host cell. Embedded within this lipid envelope are the viral glycoproteins E1 and E2, which play critical roles in virus attachment and entry into host cells. The genome itself is flanked by untranslated regions (UTRs) at both ends, which are essential for viral replication and translation. The 5' UTR contains an internal ribosome entry site (IRES) that facilitates cap-independent translation of the viral polyprotein.

HCV's lifecycle begins with the attachment of the virus to the host cell surface. This process is mediated by the viral glycoproteins E1 and E2, which interact with a variety of host cell receptors, including CD81, scavenger receptor class B type I (SR-BI), claudin-1, and occludin. These interactions are crucial for the virus to enter the host cell. Once bound to the host cell surface, HCV undergoes clathrin-mediated endocytosis, leading to the internalization of the virus into an endocytic vesicle. The acidic environment within the endosome triggers the fusion of the viral envelope with the endosomal membrane, releasing the viral RNA into the cytoplasm.

Following entry, the viral RNA is translated into a single polyprotein by the host cell's ribosomes. This polyprotein is then cleaved by both host and viral proteases into individual structural and non-structural proteins. The structural proteins include the core protein, which forms the viral nucleocapsid, and the envelope glycoproteins E1 and E2. The non-structural proteins, designated NS2 to NS5B, are involved in various aspects of the viral lifecycle, including RNA replication, polyprotein processing, and virion assembly [8].

The replication of HCV RNA occurs in a specialized membrane structure known as the membranous web, which is derived from the host cell's endoplasmic reticulum (ER). The viral non-structural proteins, particularly NS3/4A, NS4B, NS5A, and NS5B, play pivotal roles in the formation and function of this replication complex. NS5B, an RNA-dependent RNA polymerase, synthesizes a negative-strand RNA intermediate, which serves as a template for the production of new positive-strand viral genomes. These newly synthesized genomes can be translated to produce more viral proteins or packaged into new virions.

HCV assembly and release involve the coordinated action of viral structural proteins, non-structural proteins, and host cell factors. The core protein associates with lipid droplets within the host cell, serving as a scaffold for the assembly of new virions. The viral envelope glycoproteins E1 and E2 are incorporated into the nascent virions

as they bud into the lumen of the ER, forming enveloped particles. These particles are then transported through the host cell's secretory pathway and released from the cell via exocytosis.

The mechanisms of HCV infection and replication are intricately linked to various host cellular factors. The virus exploits numerous host proteins and pathways to facilitate its lifecycle and evade the host immune response. For instance, the host protein cyclophilin A interacts with the viral protein NS5A, enhancing viral RNA replication. Additionally, HCV manipulates the host's lipid metabolism to create a favourable environment for viral assembly. The viral protein NS5A interacts with host factors involved in lipid droplet formation, such as diacylglycerol acyltransferase-1 (DGAT1), promoting the production of lipid droplets that are essential for virion assembly [9]. HCV also modulates the host's immune response to establish a chronic infection. The virus employs multiple strategies to evade detection by the host's innate immune system. For example, the viral protein NS3/4A cleaves the host adaptor protein MAVS, which is involved in the production of type I interferons in response to viral infection. By disrupting this signalling pathway, HCV impairs the host's antiviral response, allowing the virus to persist and replicate within the host.

The host cell factors involved in the HCV lifecycle are diverse and play critical roles at various stages of infection. The viral entry process, for example, relies on several host receptors and co-receptors. CD81, a tetraspanin protein, is one of the primary receptors for HCV, and its interaction with the viral glycoprotein E2 is essential for viral entry. SR-BI, a lipid transporter, also plays a significant role in the initial attachment and entry of HCV into the host cell. Additionally, tight junction proteins claudin-1 and occludin are required for the virus to traverse the tight junctions between hepatocytes and establish infection.

Once inside the host cell, HCV hijacks the host's translation machinery to produce viral proteins. The IRES within the 5' UTR of the viral RNA allows for cap-independent translation, enabling the virus to efficiently produce its polyprotein even under conditions where host cap-dependent translation is inhibited. This mechanism is particularly advantageous during the host's antiviral response, which often involves the shutdown of cap-dependent translation as a defence strategy.

The replication of HCV RNA is heavily dependent on host lipid metabolism. The virus induces the rearrangement of host cell membranes to form the membranous web, a specialized compartment where viral RNA replication occurs. The formation of this structure is facilitated by the viral protein NS4B, which induces membrane alterations, and the host's lipid synthesis pathways. HCV infection is known to upregulate the expression of genes involved in lipid biosynthesis, such as those encoding fatty acid synthase (FASN) and sterol regulatory element-binding proteins (SREBPs). These changes in lipid metabolism create a lipid-rich environment that supports the formation and function of the viral replication complex.

The assembly and release of new HCV virions also involve critical host factors. The viral core protein targets lipid droplets within the host cell, which serve as assembly platforms for new virions. The interaction between the core protein and lipid droplets is mediated by the host protein DGAT1, which facilitates the association of the core protein with these lipid-rich structures. The nascent virions acquire their envelope by budding into the ER,

incorporating the viral glycoproteins E1 and E2. The assembled virions are then transported through the host cell's secretory pathway and released from the cell via the Golgi apparatus and exocytosis [10].

HCV's ability to manipulate host cell factors and evade the immune system is a key aspect of its pathogenesis. The virus employs several strategies to inhibit the host's innate immune response, allowing it to establish a chronic infection. The viral protein NS3/4A, for example, cleaves the host protein MAVS, disrupting the signalling pathway that leads to the production of type I interferons. This impairment of the host's antiviral response enables the virus to persist and replicate within the host. Additionally, HCV can modulate the host's adaptive immune response by altering the function of immune cells, such as dendritic cells and T cells, further contributing to the establishment of chronic infection.

Current Therapeutic Treatments

The treatment of Hepatitis C Virus (HCV) has significantly advanced over the past several years due largely to the development of direct-acting antivirals (DAAs). These direct-acting antiviral agents have changed the landscape of HCV therapies; these are drugs interfere with specific viral proteins required for replication and survival. DAAs have revolutionised the treatment of HCV converting it from a chronic, life destroying disease to a simple cure. DAAs act through three mainly classes that disrupt different steps on the viral lifecycle: NS3/4A protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors.

The viral protease enzyme, NS3/4A, responsible for cleaving the viral polyprotein into essential proteins required for HCV replication, is the target of NS3/4A protease inhibitors. These drugs stop the virus from replicating by inhibiting its protease, which is necessary for processing polyprotein. Non-structural protein 3/4A protease inhibitors like simeprevir, paritaprevir. NS5A is essential for both viral RNA replication and virion assembly, being the target of the NS5A inhibitors such as ledipasvir and daclatasvir. By inhibiting NS5A, they disrupt the replication process and in turn virion production. NS5B: NS5B polymerase inhibitors (for example, sofosbuvir) target the viral RNA-dependent RNA polymerase of the hepatitis C virus, which is essential for viral RNA synthesis. Sofosbuvir, a nucleoside analogue, is a chain terminator by virtue of its binding to the viral RNA-dependent polymerase. The metabolite of sofosbuvir was subsequently shown to inhibit HBV DNA synthesis in vitro.

The SVR rates of DAAs are very impressive with cure rates (SVR) above 90% in a large number of cases. The trials of these agents reveal that high efficacy rates - up to 95%, are more common - across all HCV genotypes and patient populations, including those with cirrhosis or co-infections such as HIV. The DAAs are generally well tolerated and have fewer side-effects than the older treatment regimens. DAA-based therapies are given for 8-12 weeks, depending upon the specific novel compound used and HCV genotype, and cirrhosis or other comorbidities.

Although DAAs have been successful, they have their limitations. A major challenge is the prohibitive cost of these drugs, which can impede access especially in low- and middle-income countries. Its high cost has created a myriad of treatment access issues, with many having no ability to pay for the treatment. The availability of

generic drugs and international negotiations have been pushed to reduce costs, which has had some impact but the key issue is cost. However, DAAs have high cure rates; they do not prevent reinfection. HCV-cured individuals remain susceptible to HCV re-infection. This highlights the importance of continued prevention and screening despite highly effective treatments.

DAAs furthermore have the disadvantage of treatment resistance, which is yet another limitation. While RAVs more rapidly emerge than with older therapies, may be reduced by better adherence and complete viral suppression. These resistant strains can compromise the effectiveness of subsequent treatments and demand administration of alternative drug regimens. Currently, research efforts are concentrating on delineating the underlying resistance mechanisms and design of counterstrategies such as combination therapies using DAAs that target various viral proteins.

Before DAAs, immune-based therapies have also had an important role in HCV treatment. For many years, interferon therapy was the cornerstone of HCV treatment. The type of cytokine interferons are which improve the immune reaction to viral infections. Peg-IFN is a longer half-life formulation of standard interferon and frequently used in combination with ribavirin (an anti-viral that potentiates interferon). This double therapy could reach SVR rates that varied from 40 to 50%, dependent on the patient population. Despite the effectiveness of interferon-based therapy, severe side effects (e.g., flu-like symptoms, depression, and hematologic abnormalities) preclude more general use. It is difficult for many patients to take complete this regimen because of the duration which was much longer at 24-48 weeks and the cure rates were not better, it did influence those most likely to achieve sustained virologic response.

Although the mainstay of HCV treatment has shifted toward IFN-free regimens with DAAs, immune-based therapies remain of interest. The immune response to HCV can be modulated and this property is being explored in trying to enhance the host's response against HCV, hence several studies that have evaluated immune modulators as a possible add on therapy with DAAs. Such approaches include Toll-like receptor (TLR) agonists that can activate innate immune responses or therapeutic vaccines to bolster adaptive immunity to HCV. Although experimental in nature, these strategies are at the cusp of promising advances in treating infections and preventing new ones.

Science Media Centre One of the immune modulators being assessed is a TLR7 agonist, designed to turn on immune cells, causing them to generate more anti-viral cytokines that boost the overall immune response against HCV. Previous phase one clinical trials have demonstrated that TLR7 agonists reduce the viral load and support the antiviral therapy to produce a therapeutic effect. Other lines of research cover the design of therapeutic vaccines that induce a vigorous and persistent immune response against HCV. Such vaccines are engineered to provoke both humoral (antibody-based) and cellular (T-cell-based) immunity, thereby providing a wide-range of protection against the virus. Therapeutic vaccines also have the potential to generate long-term immunity and provide durable remission and protection from reinfection, which are among the major limitations of current DAA therapies if they were successful.

In addition, studies of checkpoint inhibitors as a novel class of drugs for HCV treatment are also in the early stages; this type of drug has been successful in cancer treatment by blocking immune checkpoints that suppress T-cell activation. By blocking these checkpoints, checkpoint inhibitors work to help the immune system avoid a short circuit so it can find and knock out HCV-infected cells in the body. The strategy might be especially useful for patients with chronic HCV infection who possess an exhausted immune response.

Adoptive T-cell therapy is another very exciting class of immune-based therapies. This is administration of ex vivo stimulated and expanded autologous HCV-specific T-cells into patient. These T-cells are able to specifically target and kill HCV infected cells ultimately leading to viral clearance. This is novel approach at present time, Immune system as armament to control HCV Still in childhood but has the potential to tap the immune response in the battle against HCV.

With the advent of DAA therapy and the promise of immune-based approaches, many remaining challenges are being met end route to achieve the elusive goal of HCV eradication. Among others, screening and appropriate diagnosis was a major challenge. Thousands of those with HCV are living without knowing that they are infected and so the virus continuous to spread; it is often not diagnosed until late stage when treatment is less likely to work. Improving access to local HCV testing plus access to diagnostics would be a key driver of early diagnosis and treatment of HCV.

In addition, integrated care models to address comorbid substance use disorders and mental health conditions commonly observed with HCV are needed. Patients with HCV benefit from comprehensive care that includes behavioural health services, substance use treatment and social support, which can lead to improved adherence to antiviral therapy and other health outcomes.

Targeting Host-Virus Interactions

Investigation of these (host-virus) interactions offers an exciting and unexplored vista in the developments of novel therapeutic strategies against HCV. This was important: in reflection these interactions are the basic mechanism as to how HCV actually infects a hepatocyte, replicates, and escapes immune surveillance. By inhibiting these interactions, it is perhaps feasible to severely impair the infectious lifecycle resulting in improved and lasting antiviral approaches.

Life cycle The life cycle of HCV is initiated by the entry of the virus into host cell and encompasses a coordinated series of complex events-viral glycoproteins mediate binding to specific host cell receptors. Viral envelope glycoproteins (E1 and E2) bind to the surface of host cell. The glycoproteins bind to multiple host cell receptors including CD81, scavenger receptor class B type I (SR-BI), cljsonin-1, and occludin. The tetraspanin protein CD81 is a key molecule in the early attachment of HCV to the host cell. In general, SR-BI is a lipid transporter which supports viral entry by interacting with the E2 glycoprotein, promoting virus binding and fusion. HCV crosses the tight junctions between hepatocytes using cell-cell junction by claudin-1 and occludin (but not JAM-A), both of which are tight junction proteins, to undergo its entry to establish infection.

HCV enters the cell following attachment and initiates a series of intracellular signalling events that result in viral replication and establishment of persistent infection (Doherty and Brotchie, 2005; Fiorucci et al., 1998). Here, we show that TssK is a secreted protein transported to host cells, where it may subvert host signalling; the serine protease activity of its paralog TagF also enables this species to arrest the phagolysosome and appear in autophagosomes, two steps in bacterial escape from lysosomal destruction These processes are linked by TssK-mediated activation of the host's phosphoinositide 3-kinase (PI3K)/Akt signalling pathway Such redundancy illustrates how human ubiquitin-based immune responses might be met i.e,escaped by multiple strategies. When viruses gain control of this pathway, they can maintain the survival state and suppress apoptosis in virus-infected cells to create a situation favourable for viral production. NS5A and other HCV proteins interact with components of the PI3K/Akt pathway, promoting activation of downstream Akt effector molecules that Favor viral replication and persistence.

The other key host-virus interaction occurs at the ER level where HCV setups its replication complex. The membrane-associated viral non-structural proteins NS3/4A, NS4B, NS5A and NS5B control the formation and function of the viral replication complex or replicase that localizes to an unique membrane structure called replicative organelles or the "membranous web" 1. The double-membrane structure is formed post-replication and is composed of proliferated host ER, suggesting widespread re-organization within the membrane (Fig. NS4B triggers the formation of these membrane constructions that in turn allows a proficient cytoplasmic refuge for viral RNA replication. NS5A (non-structural protein 5A), a little known but highly multifunctional (go to section n, below) protein makes contacts with a large number of host factors which participate in the same processes of lipid metabolism and membrane trafficking activities that aid and abet stability as well as function of the replication complex. Within this complex, NS5B (the RNA-dependent RNA polymerase) is responsible for replicating new viral RNA genomes to produce progeny virions [11].

There are several advantages to developing host-targeted inhibitors rather than directly targeting viral proteins using traditional antiviral strategies. This lowers the likelihood of resistance development, one of the main advantages. The viral proteins, especially the ones which are play a major role in replication have tendency to mutates at a fast pace hence resistant strains emerge. On the other hand, potential host factors implicated in viral entry, replication and assembly are usually conserved due to their role in normal cellular functioning. Targeting these relatively stable host factors is thought to limit the emergence of resistance, which could result in longer-lasting antiviral responses.

In addition, host-virus running blocks may, by definition, possess broad Spectrum antiviral activity. A myriad of viruses hijack the same cellular machinery and host pathways to replicate and thrive (22). Thus, pharmacological agents that target broad host-virus interactions could treat diverse viral infections. For instance, the PI3K/Akt pathway is critical for both HCV and lipopolysaccharide replication, and molecules interfering with this pathway may not only dampen HCV virus production ( ) but also decrease that of other viruses like HBV ( ). In many ways, these drugs may mimic the activity of inhibiting host lipid metabolism pathways that direct the formation of the

viral replication complex depending upon their role in stimulating a broad spectrum of lipid-dependent viruses [12].

An exciting method for targeting host-virus interactions is by small-molecule inhibitors designed to selectively perturb the interaction of viral and host cell proteins. For example, some small molecules targeting the E2 glycoprotein of HCV could inhibit the binding of HCV to its receptor CD81 or SR-BI to prevent viral entry and thereby limiting infection establishment [9]. Entry inhibitors thus be administered prophylactically to prevent HCV transmission or therapeutically to suppress viral load in infected individuals. Moreover, blocking viral entry and neutralizing the virus can be achieved by employing or designing monoclonal antibodies directed towards host receptors and also towards viral glycoproteins.

An alternative strategy is to target host cellular pathways manipulated by HCV for its replication (CCR8). For instance, agents targeting the pro-survival signals triggered by HCV via PI3K/Akt pathway can decrease viral replication and promote apoptosis of infected cells. In the same way inhibiting the viral replication complex by targeting host components required for assembly and functioning of it, such as enzymes involved in lipid metabolism or membrane-trafficking proteins, will result in reduced production of new virions because the virus is unable to replicate. For example, uncovering drugs that inhibit the interaction between NS5A and host lipid droplets can block new virions from being produced, thereby decreasing viral load [13].

An obstacle in the field of host-virus interface is the necessity to inhibit precisely and to avoid side effects. This is particularly important in the context of biology, since these host factors participate in multiple cellular processes and consequently inhibiting them could have collateral damages leading to side-host responses and toxicity. Consequently, a detailed consideration of the direct mediate interactions between HCV and host factors is indispensable for the identification and development of such targeted therapies that specifically block viral functions while retaining cellular homeostasis. Thanks to developments in high-throughput screening and structural biology, we now know of many critical host-virus interactions and have the capacity to develop selective inhibitors with few off-target effects.

Aside from small molecule inhibitors, another attractive target for anti-VACV strategies are the host factors that mediate VACV-host interactions, via technologies such as RNA interference (RNAi). RNA interference (RNAi) that can shut down host cell genes essential for viral replication by small interfering RNA (siRNA). Small interfering RNA (siRNA) selectively targeting and knocking down these host factors might efficiently suppress HCV replication. In preclinical studies this approach has shown promise, and efforts are ongoing to improve the delivery and stability of RNAi-based therapeutics in order to improve their clinical efficacy.

Another novel approach, Consisting of the use of CRISPR/Cas9 gene-editing technology to knockout essential host-altered genes for HCV replication. CRISPR/Cas9 is an ideal system to ensure a durable treatment with the possibility of cure by specifically targeting and modifying these genes. This technology is also unique because it allows to target multiple host factors at once which results in a multifaceted attack on the viral lifecycle.

Exploiting Host-Virus Interactions as Novel Therapeutic Approaches

Antiviral therapy has made significant strides in the fight against Hepatitis C Virus (HCV), but drug resistance remains, and a more comprehensive response is still required. Developing HTTs (host-targeted therapies) is an innovative strategy that has attracted public interest. In stark contrast to traditional antiviral drugs, which attack the virus itself - like Tamiflu or flu shots during the flu season - HTTs work against hepatitis C by interrupting just how much the virus relies on hijacking existing biological pathways inside the host cell in order to replicate.

Host-targeted therapies act by targeting host cell factors and networks that the virus utilizes to replicate itself and establish a state of persistence. The rationale is that because host cell factors are genes with low genetic stability, limited resistance will arise compared to the evolving viral proteins. With regard to circumventing antiviral resistance, this strategy is advantageous in that it can provide broad-spectrum antivirals targeting multiple viruses depending on the similar host factors [14].

A particular area of growth in HTTs is the use of small-molecule inhibitors that target pertinent host cell receptors and enzymes. For instance, PI3K/Akt signalling pathway has been shown as a potential target [14]. HCV exploits this pathway to establish an environment in which it can replicate. PI3K inhibitors will be tested with the goal to interrupt this aberrant signalling pathway, ultimately suppressing viral replication. Inhibitors of this sort have shown the ability to reduce HCV RNA levels within infected cells, indicating that this approach could be potentially successful.

A third attractive HTT strategy entails the manipulation of host lipid metabolism, which essential targets for HCV replication complex assembly. The virus causes massive remodelling's of the endoplasmic reticulum from the host cell to form a membranous web in which the replication complex resides. For example, FASN inhibitors as small molecules that disrupt the biosynthesis of lipids effects cellular architecture: viral replication needs to form several structures that could be blocked by FASN inhibitors. This approach has also demonstrated better results in preclinical studies, suggesting its viability for therapeutic purposes.

The fact that there may be dozens of different host-targeted therapies currently in development is a testament to the variety of approaches that are being considered. Alisporivir, a cyclophilin inhibitor aimed at host protein required for HCV replication and assembly. Typically, such interventions are first evaluated in clinical trials for their ability to reduce viral load without the emergence of high-level resistance. Even so, silibinin, a flavonolignan from milk thistle inhibiting HCV entry and replication by addressing host cell receptors and signalling pathways

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The concept of host-targeted antiviral therapy using already approved drugs is also emerging. The researchers also detailed that cellular pathways which are exploited by viruses can be targeted by many drugs and these drugs often have been developed for other indications. For example, statins, classy of molecules know to reduce cholesterol levels, have been explored to inhibir lipid metabolism pathways necessary for HCV replication [19]. More recent clinical studies have revealed that their addition to DAAs significantly improved the antiviral effect of DAAs, resulting in a greater reduction of viral load than treatment with DAAs alone.

Metformin, a typical diabetes molecule offers, " in drug repurposing. There is evidence indicating that AMP-activated protein kinase (AMPK) can inhibit viral replication by altering metabolic pathways, and studies have recently shown that metformin activates the AMPK in mammals [9]. Metformin has been demonstrated to inhibit HCV replication in vitro and clinical trials are ongoing to assess its efficacy in HCV-infected patients. The main benefit of using approved drugs is the long list of safety and pharmacokinetic data that can facilitate much faster clinical development [15].

The success of HTTs hinges on the validation of both their safety and efficacy in clinical trials. A few HTTs have currently entered into clinical trials, and some have shown promising results. For example, a Phase I assessment of the FASN inhibitor TVB-2640 in HCV-infected patients showed marked viral load decreases and was well-tolerated [185]. Similarly, alisporivir, a compound which is an inhibitor of cyclophilin, has exhibited strong antiviral activity in clinical studies and a low potential for resistance development.

RNAi Based Approaches Another advanced methodology in host evasion is depletion of host virus interactions employing RNA interference (RNAi). RNAi consists of small interfering RNAs (siRNAs) that silence the expression of host genes necessary for viral replication. RNAi can decrease viral load and slow down the progress of an infection by silencing these host genes. Despite the great progress that has been made in the RNAi therapy field, one of the concerns in developing siRNAs as HCV therapies is that liver, being the major organ target for HCV replication, where emerging of sufficient levels and duration covering adequate time from a single or repeated injection delivered siRNA remains a particularly challenging issue.

To combat this, researchers are designing improved delivery vehicles to improve the stability and specificity of siRNAs. Lipid nanoparticles (LNPs) have been particularly useful for delivery of siRNAs and to increase their intracellular uptake by hepatocytes. LNPs shield siRNAs from blood degradation and enable their delivery to the target cells. Overall, human clinical trials using LNP-formulated siRNAs have shown promise with marked reductions in HCV RNA levels in patients.

Another clever delivery strategy entails using viral vectors, like adeno-associated viruses (AAVs), to shuttle siRNAs into the liver. AAVs were selected because they could penetrate hepatocytes efficiently and sustain the expression of siRNAs for therapy. Preclinical studies have demonstrated that AAV mediated delivery of siRNAs against HCV host genes reduces viral load efficiently. Current research is aimed at enhancing these delivery systems to increase the efficacy and safety of targeted treatment in a clinical setting.

RNAi strategies are very promising; however, to achieve their full potential as therapeutics, a number of issues must be resolved. SiRNAs can also have off-target effects in which the siRNA inadvertently silences nontarget genes. While the effects of these off-targets will vary by gene target and cell line, advances in bioinformatics and siRNA design algorithms are helping to ameliorate this issue by the increased specificity of siRNAs. In addition, immune responses to the delivery vehicles including LNPs or viral vectors must be managed to ensure safety and efficacy of the therapy.

In addition, IFN-free regimens using DAA drugs empty the way to possible co-administration of RNAi strategies that coupled with novel antiviral molecules or cohorts may help enhance outcomes. For instance, exploiting RNAi to target cellular factors required for viral replication in concert with directly acting antivirals (DAAs) that inhibit viral proteins would be synergistic, providing the opportunity to decrease the IC50 and likelihood of resistance. Such a combination strategy is under investigation in preclinical and early phase clinical studies and shows promising efficacy.

Conclusion

While the development of direct-acting antivirals (DAAs) has made significant strides toward combating hepatitis C virus (HCV), challenges including drug resistance, toxicity and the ability of the virus to establish persistence in certain populations highlight an urgent need for alternative therapeutic strategies. Targeting host-virus interactions provides a novel approach to antiviral drug discovery, and preferably one in which the target is a host cellular factor that does not change through viral evolution due to its stable and essential nature in the cell. During the course of HCV replication, many host factors or pathways that are utilized at different stages can be targeted for drug development. Traditional antivirals directly affect viral proteins and are therefore susceptible to resistance resulting from the high mutation rate of all RNA viruses where drug action is mediating by inhibiting viral replication by targeting directly essential enzymatic function in contrast HTTs target cellular factors which are more conserved among RNA viruses and have limited ability to mutate into resistant strains so leading reduced potential for inducing escape mutants. In addition to healing tissue, this could provide a more transient therapeutic solution but has the added potential for broad-spectrum antiviral activity as many viruses rely on common host-dependent pathways and mechanisms. For example, inhibition of the phosphoinositide 3-kinase (PI3K)/Akt signalling pathway by small-molecule inhibitors or disturbance of host lipid metabolism necessary for viral replication have proven effective in preclinical studies and early clinical trials. In addition, repurposing existing drugs adds a practical aspect to this strategy since we already have abundant safety and pharmacokinetic data on these drugs. Statins and metformin represent the most striking examples in which drugs devoted to cardiovascular/metabolic disorders have shown antiviral activity against HCV via endogenous intermediates blockade. RNA interference (RNAi) therapies, designed to target host genes critical for viral replication, offer a different strategy in this creative therapeutic field. The recent breakthroughs in the delivery of RNAi such as lipid nanoparticles (LNPs) and viral vectors have developed great potentials to stabilize and target RNAi, which represent a major step forward in launching RNA interference into clinical practice. Last but not least, CRISPR/Cas9 gene-editing technology is about to revolutionize the manner by which host genes that are important for HCV replication can be targeted and modified, thereby offering a potential curative approach. Combining these new NS3/4A HCV protease inhibitors with existing DAAs could result in additive or synergistic benefits and may reduce resistance issues. Nevertheless, the road to fully unlock host-targeted therapies is a minefield. Key concerns about these therapies are to ensure their specificity and not cause off-target effects

while it may also affect normal cellular functions. In addition, the specific needs around regulation and clinical trial environment for these unique therapies must be carefully addressed to ensure their safety, efficacy and translation among our diverse population. With host-targeted therapies, regular monitoring to detect any resistance development is still required, and this is as important as it seems in maintaining their effectiveness in the long term. Personalized medicine approaches are required to optimize the benefits and minimize the risks of these therapies, partly due to their complexity. As research progresses on the use of newly introduced technologies for a personalized medicine approach to HCV treatment, it is apparent that these emerging technologies fit into the new landscape for both more effective and sustainable eradication strategies. Finally, host-virus interactions as a drug-devoting strategy for HCV infection constitutes a new change of conducing treatment against HCV instead of using these very poor management practices allowing us to approach the aim of eradication. Ongoing investment in research and development is needed to realise the promise of these innovative strategies and ensure they benefit for all people living with HCV by delivering safe, effective and accessible treatments. This combination of HTTs, repurposed drugs, RNAi and recent gene-editing technologies illustrates the multi-faceted avenues that not only mark a new era in HCV control but signals potential future directions for antiviral targeting strategies more broadly. The hard path forward is filled with promise when it comes to reshaping the antiviral armamentarium and global health for tomorrow.

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