Hepatitis C Virus and Antiviral Treatment

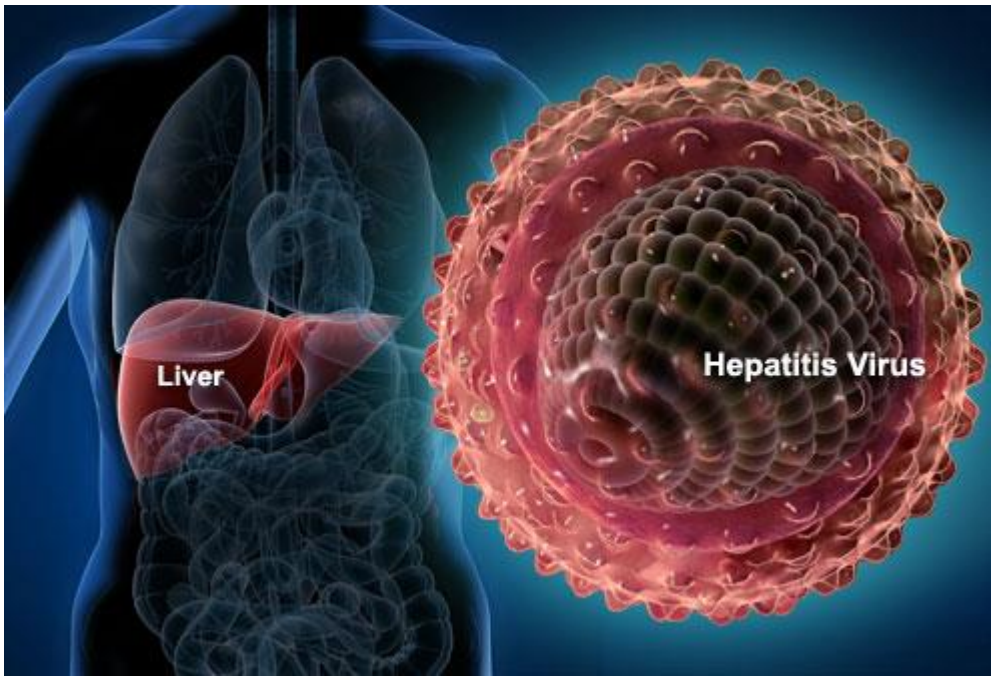

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Figure obtained from WebMD.com

Abstract

Worldwide, around 200 million individuals are infected with hepatitis C virus (HCV) and every year 350.000 persons die because of the consequences of HCV. HCV infection is therefore determined to be a serious global public health problem. Standard of care therapy has limited efficacy and is associated with multiple frequent and serious side effects. Thanks to available information about the molecular biology of the virus, the development of direct-acting treatment against the HCV non-structural proteins NS3, NS4A, NS5A has expanded for the first time since the discovery of HCV. However, the newly developed treatments still contain several limitations, including low genetic barrier to viral resistance, adverse effects, drug tolerability by the virus. Another concern is that HCV will never be eradicated by antiviral drugs since reinfection can occur after antiviral treatment. Another not insignificant problem of antiviral treatment are the very high, and even in most of the world, unaffordable costs. In this essay, the standard of care therapy, newly developed antiviral treatments and eventually, future perspectives of these antivirals are discussed.

Table of Contents

Introduction

In 1989 it was discovered that hepatitis C virus (HCV) is the cause of non-A-hepatitis and non-B-hepatitis (Tosone et al., 2014, Kim et al., 2013). Since then, HCV is determined to be a serious global public health problem. Almost 10 years ago, the World Health Organization estimated the prevalence of HCV (Shepard et al., 2005). 2,2-3% of the world population is infected with this virus, which is around 200 million people. Every year, more than 350.000 individuals die because of HCV (Shahid et al., 2014). The highest reported prevalence rates are found in countries located in Africa and Asia (Shepard et al., 2005), as shown in figure 1.

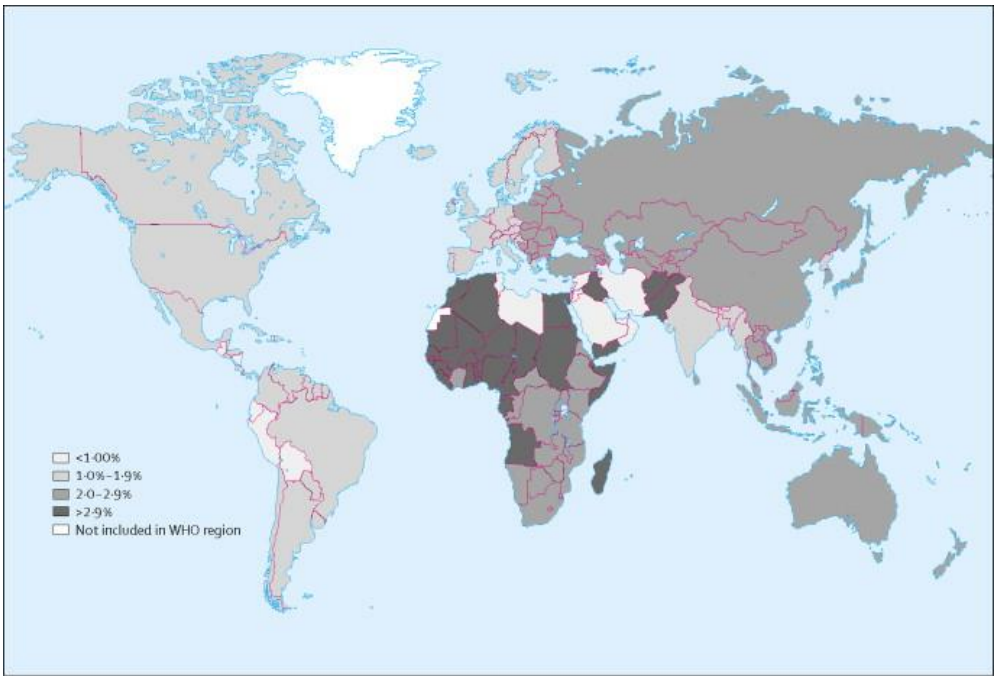

Figure 1 Global prevalence of Hepatitis C virus (HCV) infection reported by World Health Organization. Worldwide, HCV-infected individuals are registrated. However, there is a large degree of geographic variability in the distribution of HCV infection. Highest prevalence rates are registered in countries in Africa and Asia (dark grey and darker grey). Countries with lower prevalence rates are located in northern and western Europe, north America, South America and India (light grey) (Figure obtained from Shepard et al., 2005).

HCV is a blood-borne virus and transmission of HCV can therefore occur through contact with infected blood, sharing of infected injection equipment for drug use, medical practices in which medical equipment is reused or inadequate sterilized. HCV can also be transmitted from an infected mother to her baby. Sexual transmission also occurs (Lacombe, 2014, Sebastiani et al., 2014).

HCV can cause acute and chronic infection. Acute infection of HCV is very often asymptomatic and therefore often not recognized clinically. Acute HCV infections in most cases do not resolve spontaneously (Clark & Nelson, 2009). In the majority of the patients (80%), the viral infection persists and chronic HCV infection develops (sebastiani et al., 2014, Ip et al., 2012).
The innate as well as the adaptive immune responses fail to clear the viral infection. The virus possesses efficient strategies to escape from the immune responses, resulting in viral persistence (Thimme et al., 2012). Not only the fact that the hepatitis C virus possesses strategies to escape the immune system plays a pivotal role in persistence of the viral infection. It is found that the HCV-specific $CD8^+$ T cells isolated from chronic infected patients have an impaired effector function resulting in inefficient clearance of the virus (Wedemeyer et al., 2002). Thus also impaired effector functions of the immune system play a major role in the inefficient clearance of the virus, resulting in the development of chronic HCV infection. Chronic HCV infection can lead to the development of

severe liver disease including cirrhosis and hepatocellular carcinoma. In some cases, liver cirrhosis caused by chronic HCV infection affects the liver such that liver transplantation is needed. Even extra-hepatic complications, such as lymphoma, have been reported to be linked with HCV (Tosone et al., 2014). To cure HCV infection and prevent the consequences of HCV infection, treatment of HCV infection is highly important.
Since the discovery of HCV, available standard of care treatment had limited effect (Coilly et al., 2014). Recently, for the first time the development of antiviral therapies has expanded, especially the development of direct-acting antiviral treatment (Fusco & Chung, 2012). Despite, these antiviral therapies have several limitations (Shahid et al., 2014).

In this essay, I will first describe the molecular biology of the Hepatitis C virus to provide more understanding about the virus. Next, I will describe and discuss the standard of care therapy, newly developed antiviral treatments and eventually, the future perspectives of these antivirals.

Virology and life cycle

Hepatitis C virus is a small, enveloped virus containing positive sense, single-stranded RNA consisting of 9600 nucleotides. HCV is a member of the Flaviviridae family in the genus Hepacivirus (Ip et al., 2012, Kim et al., 2013). The HCV genome consists of one open reading frame which encodes a polyprotein build of 3000 amino acids which is flanked by 5' and 3' untranslated regions (UTRs). Viral particles are lipidated with LDL and VLDL when circulating in the human body. Via these lipoproteins, the virus can bind to several receptors on the hepatocyte cell surface allowing entrance of the virus particle through endocytosis. Receptors identified to be involved in HCV endocytosis are tetraspandin CD81, scavenger receptor class B type 1, occluding and claudin-1 (Ip et al., 2012, Lin et al., 2014).
Following receptor-dependent endocytosis, the viral RNA genome is uncoated and released in the cytoplasm (Kim et al., 2013). Here, host ribosomes translate the viral RNA genome into a single polypeptide (Lin et al., 2014). Subsequently, the polypeptide is cleaved posttranscriptionally by viral as well as host proteases resulting in the production of 10 viral proteins. These 10 proteins include the structural proteins C, E1 and E2, and the non-structural (NS) proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Fusco & Chung, 2012). The translation and posttranslational cleavage into the 10 viral proteins is illustrated in figure 2.

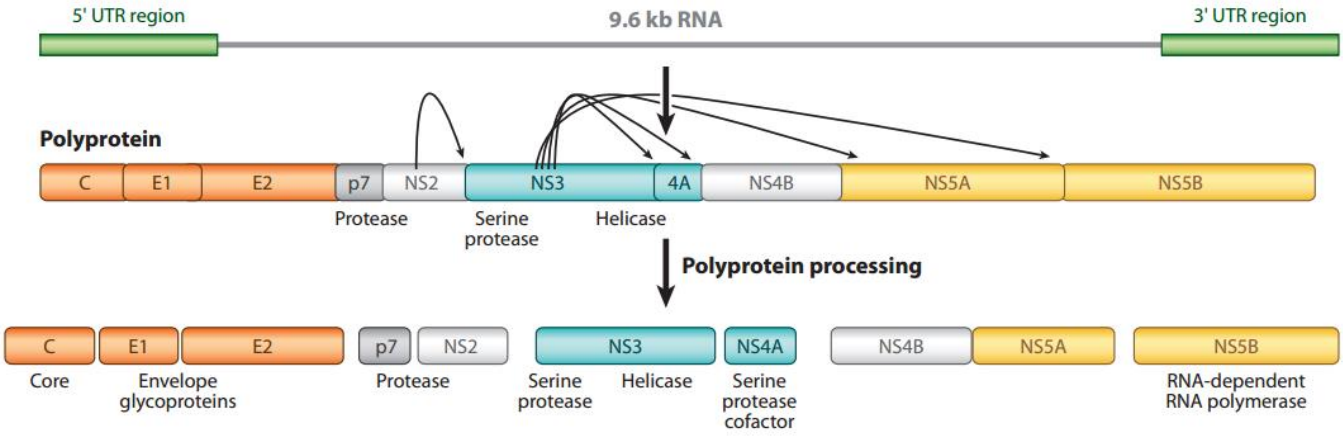

Figure 2 The translation of the HCV genome and the posttranscriptional cleavage of HCV peptide in 10 proteins. The RNA genome of HCV consisting of 9600 nucleotides and at the 5' and 3' end an UTR region, is translated in one polypeptide. Posttranscriptionally, this peptide is cleaved into 10 viral proteins, including the structural proteins protein C (core protein), E1 and E2 (envelope glycoproteins), p7 and NS2 (proteases), NS3 (serine protease and helicase), NS4A (serine protease cofactor), NS4B and NS5A, and eventually NS5B (RNA-dependent RNA polymerase). The NS3/NS4A complex cleaves the polyprotein at four sites (arrows from NS3 to the cleavage sites) (Figure obtained from Fusco & Chung, 2012).

The structural proteins, protein C and the envelope proteins E1 and E2 serve to assemble the newly formed viral particles, whereas the non-structural proteins are vital for replication, polyprotein processing and propagation of the virus (Lin et al., 2014, Saeed et al., 2013). Below, the functions of non-structural proteins NS3, NS4A, NS5A and NS5B are discussed, since these proteins are targets for treatment of HCV infection (Kronenberger & Zeuzem, 2012). Inhibitors of these non-structural HCV proteins are already developed and studied, as will be described in the next chapter.
NS3 is a serine protease and belongs to the trypsin/chymotrypsin superfamily. NS4A is the cofactor of NS3 and together they can form the NS3/4A complex which exerts protease activities. The NS3/4A complex catalyses the cleavage of the viral polyprotein at four sites. The polyprotein is cleaved between NS3, NS4A, NS4B, NS5A and NS5B (Shahid et al., 2014), which is also shown by the black arrows on top of the polyprotein in figure 2.

NS5A is a regulatory, non-enzymatic phosphoprotein found in phosphorylated and hyperphosphorylated forms (Gerold & Pietschmann, 2014, Dhingra et al., 2014, Moradpour et al., 2007). Ascher and colleagues reported that NS5A binds to the 3'UTR of the newly formed RNA.
Here, NS5A plays a role in viral RNA replication partially through the interaction with viral RNA-dependent RNA polymerase NS5B (Ascher et al., 2014). The NS5A is thus essential for HCV replication, assembly and propagation. However, others state that the function of the NS5A protein in not exactly known (Dhingra et al., 2014, Belda & Targett-Adams, 2012).
NS5B is the RNA-dependent RNA polymerase of HCV and replicates RNA required for new virus particles (Marascio et al., 2014). During replication, the NS5B RNA-dependent RNA polymerase uses the positive sense RNA genome as template to generate negative sense RNA intermediates. These negative sense RNA intermediates are subsequently used to generate new positive stranded genomes (Kim et al., 2013).

The replication of the viral genome occurs in a so-called 'membranous web', which is an endoplasmic reticulum membrane–derived replication complex including double-membrane vesicles. These vesicles contain viral RNA, ER membranes, lipid droplets and the non-structural viral proteins NS3, NS4A, NS4B, NS5A, and NS5B (Kim et al., 2013, Schaefer & Chung, 2012). The newly synthesized HCV RNA is packed in new viral particles. Subsequently, the hepatocytes release the viral particles (Kim et al., 2013). A schematic overview of the HCV life cycle and replication is shown in figure 3.

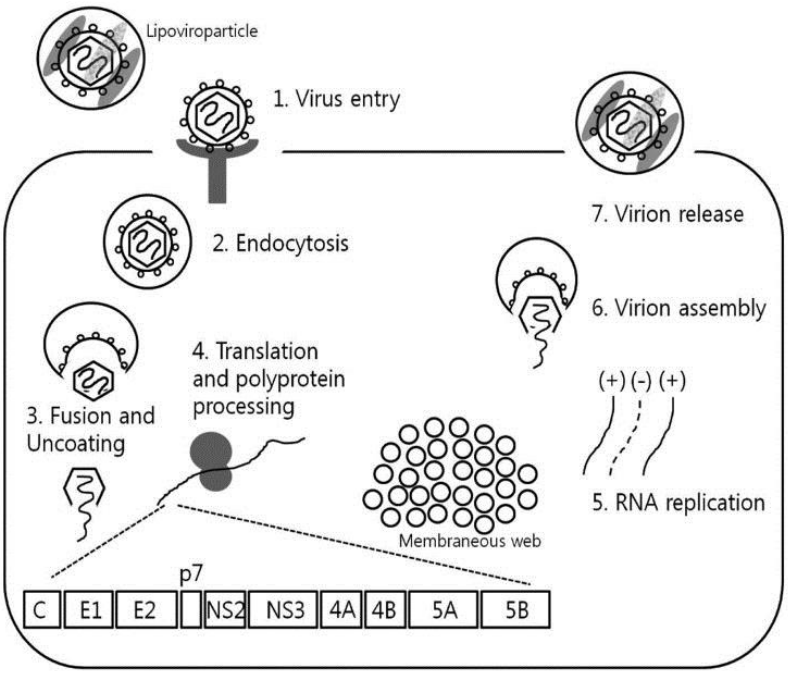

Figure 3 Hepatitis C virus life cycle and replication. The HCV particle is surrounded by lipoproteins, together called the lipoviroparticle. This viral particle binds to receptors on the hepatocyte cell surface (1). Due to the engagement with the cell surface receptors, the viral particle is entering the cell through endocytosis (2). The membranes of the viral particle and the endocytosis vesicle fuse, resulting in uncoating of the viral RNA and nucleocapsid (3). The viral RNA is translated into the viral polypeptide by host ribosomes and posttranscriptionally cleaved by host and viral proteases (4). The viral RNA is replicated in the membranous web (5) and hereafter the newly synthesized RNA is assembled into new lipoviral particles (6). Eventually, the virus particle is released by the cell (7). (Figure obtained from Kim et al., 2013).

In patients with chronic HCV, the virus produces approximately 10^{12} viral particles each day. Due to this rapid viral replication and the high error rate of NS5B RNA dependent RNA polymerase, which is approximately 1.92 x 10^{-3} base substitutions per site per year, nucleotide mis-incorporations accumulate (Ip et al., 2012). These mutations give rise to genetic diversity in HCV. Already 7 major genotypes are determined which are classified in 67 subtypes (Smith et al., 2014). Genotypes 1, 2, 3 and 4 are most common, whereas genotype 7 is extremely rare (Feeney & Chung, 2014). Due to this

genetic variability, the replication of the virus improves, mutants are established escaping the host immune system. Hereby the virus is not recognized and cleared and the virus also gains persistence to antiviral therapy whereby treatment of HCV is difficult (Cox et al., 2005, Lin et al., 2014). The treatment of HCV infection is therefore dependent on the genotype of HCV the patient is infected with. Genotyping is thus needed to determine the type and duration of the treatment (Feeney & Chung, 2014).

Anti-viral treatment

Currently, antiviral treatment is used to treat HCV. The goal of the treatment is to achieve viral clearance, also called sustained virologic response. This is defined as no detection of HCV RNA in the patients 24 weeks after completion of treatment (Feeney & Chung, 2014). Below, the different treatments against HCV are explained.

Indirect-acting antivirals

Even before the identification of HCV in 1989, interferon-α had already been shown to have beneficial effects in chronic HCV (Hoofnagle et al., 1986). Since then interferon-α has been used as treatment against HCV infection. Interferon-α is a protein present in the human body produced after infection (Hoofnagle et al., 1986, Feeney & Chung, 2014). Interferon-α does not directly act on the virus. However, upon engagement with its receptor, it induces the expression of interferon-stimulated genes resulting in a non-virus-specific antiviral state within the cell (Bekisz et al., 2004). Treatment with interferon-α for 6 months had only limited sustained responses of 6-12%. Therefore, interferon-α administration was combined with administration of ribavirin. Ribavirin is an oral guanosine analog with antiviral activity against several viruses, including HCV. The simultaneous administration of interferon-α and ribavirin more than doubled the sustained response rate to 35-40%. Another improvement of the interferon-ribavirin treatment was the covalent attachment of poly(ethylene glycol) to interferon-α (peg-interferon-α). Peg-interferon-α administration in combination with ribavirin resulted in better virologic responses of 54-56% (Feld & Hoofnagle, 2005). The combination of administration of interferon and ribavirin has therefore until recently been used as standard of care treatment for HCV infection (Feeney & Chung, 2014). Although the administration of peg-interferon-α in combination with ribavirin results in 54-56% virological response rates, 44-46% of the HCV infected patients do not show improvement with this therapy. The response of this treatment is also genotype dependent (Feeney & Chung, 2014). Other limitations of this therapy are the fact that this combination therapy is expensive (Feld & Hoofnagle, 2005) and it is associated with multiple side effects. Side effects are serious and include flu-like symptoms, anemia, thrombocytopenia, autoimmunity, and thyroid dysfunction (Feeney & Chung, 2014, Shahid et al., 2014). Some groups of patients who suffer from advanced liver disease or liver transplant failure are even contraindicated to treatment with Peg-interferon and Ribavirin, resulting in further limited treatment access (Shahid et al., 2014, Degasperi & Aghemo, 2014).

Direct-acting antivirals

Therefore, antiviral treatment has been developed which directly acts on hepatitis C virus proteins, unlike ribavirin and (peg-)interferon. Recently, the development of direct-acting antiviral therapies has expanded for the first time since the discovery of HCV (Fusco & Chung, 2012). In particular the non-structural HCV proteins are attractive targets for the development of direct-acting antivirals. Different types of direct-acting antivirals against HCV have already been developed, including NS3/4A inhibitors, NS5A inhibitors, and NS5B inhibitors (Feeney & Chung, 2014). Currently, multiple improved NS3/4A inhibitors, NS5A inhibitors, and NS5B inhibitors are studied (clinicaltrials.gov).

Below, the different types of direct-acting antivirals are discussed. Some already licensed antivirals, as well as some antivirals that are currently studied in clinical trials are given.

NS3/4A protease inhibitors

The NS3/4A inhibitors, also called protease inhibitors, inhibit the non-structural proteins NS3 and NS4A of the hepatitis C virus. NS3/4A protease inhibitors are the first class of direct-acting antivirals (DeLuca et al., 2014). As already shown, NS3 and NS4A together from the serine protease of the virus (figure 2) that cleaves the polyprotein at four sites (Dhingra et al., 2014). When the viral serine protease is inhibited, the viral polyprotein will not be cleaved after transcription. The virus can therefore not produce functional structural and non-structural proteins anymore, including the RNA-dependent RNA polymerase NS5B which is responsible for viral RNA replication. This results in inhibition of the viral propagation, replication and assembly (Marascio et al., 2014). NS3/4A protease inhibitors have high antiviral effects (Shahid et al., 2014).

First generation protease inhibitors were telaprevir and boceprevir, and were also the first direct acting antivirals (DAAs) (Feeney & Chung, 2014, Dhingra et al., 2014). Treatment of telaprevir or boceprevir in combination with peg-interferon and ribavirin has been shown to have high efficacy against HCV genotype 1 compared to standard of care therapy (Kim et al., 2014). Multiple other NS3/4A protease inhibitors have been investigated since the discovery of telaprevir and boceprevir (Dhingra et al., 2014).

Currently, second wave first generation NS3/4A protease inhibitors are developed and studied in clinical trials. The second-wave, first-generation NS3/4A protease inhibitor simeprevir has just been licensed (Feeney & Chung, 2014). Simeprevir is used as treatment against genotype 1 and its efficacy increases when administered in combination with interferon and ribavirin. A benefit of this antiviral is that the dose should be taken once daily, compared to two or three times per day. In combination with interferon and ribavirin, Simeprevir is very efficient (Kanda et al., 2014). At the moment, Asunaprevir and Faldaprevir are studied in phase II and phase III respectively (Kim et al., 2014). Asunaprevir can be used as treatment against HCV genotype 1 and 4. This drug has to be taken twice daily (Gentile et al., 2014). Faldaprevir, however, can be taken once daily, which is an advantage over the daily intake of Asunaprevir. HCV genotype 1 can be treated with Faldaprevir. Combination therapy of Faldaprevir and interferon and ribavirin is very efficient (Chen et al., 2014). Danoprevir has been studied in a phase I study (Canini et al., 2014). This drug is a twice daily protease inhibitor. In combination with interferon and ribavirin, Danoprevir is very efficient in treating HCV genotype 1 (Feeney & Chung, 2014). Second-generation protease inhibitors are in clinical trials (Kim et al., 2014). Resistance of HCV to protease inhibitors through resistance mutations is a common occurrence when treatment fails (Feeney & Chung, 2014, Gentile et al., 2014).

NS5A inhibitors

NS5A inhibitors have also been developed as direct-acting antivirals. NS5A is one of the proteins produced from the HCV polyprotein (Fusco & Chung, 2012) as shown in figure 2. As mentioned before, some state that the exact function of the NS5A protein in not exactly known (Dhingra et al., 2014, Belda & Targett-Adams, 2012). Others mention that the NS5A protein is essential for HCV replication, assembly and propagation (Ascher et al., 2014). Despite these inconsistencies, the NS5A inhibitors may be the most potent antivirals ever discovered (Belda &Targett-Adams, 2012). Like NS3/4A protease inhibitors, NS5A inhibitors have high antiviral efficacy (Shahid et al., 2014). Currently, multiple potent NS5A inhibitors are under investigation. A very promising and also the first approved NS5A inhibitor is Daclatasvir (Kim et al., 2014), which targets HCV genotype 1a, 1b, 2a, 3a and 4 (Lee, 2013). Combination therapy with Daclatasvir and other drugs is currently extensively studied in clinical studies (clinicaltrials.gov). Kim et al. states that combination therapy with Daclatasvir is essential for all HCV genotypes (Kim et al., 2014). The investigation of another NS5A inhibitor, Ledipasvir, just completed phase III (clinicaltrials.gov). Unlike Daclatasvir, Ledipasvir also targets genotype 6a (Gentile et al., 2014). A phase-II study with ABT-267 has also just been completed (clinicaltrials.gov). ABT-267 targets all HCV genotypes (Stirnimann et al., 2014). The drugs

tested until recently, do not show much drug-drug interactions and also the adverse effects are minimal (Feeney & Chung, 2014).

NS5B inhibitors

NS5B inhibitors inhibit the RNA-dependent RNA polymerase NS5B of hepatitis C virus, which replicates the RNA to synthesize viral progeny (Marascio et al., 2014), as stated before. Two types of NS5B inhibitors exist, namely the nucleotide/nucleoside analogue inhibitors (NIs) and the non-nucleoside inhibitors (NNIs). Nucleotide/nucleoside analogue inhibitors (NIs) bind to the active site of the RNA polymerase and cause direct termination of the RNA chain. Examples of NIs are Mericitabine (RG1728) and Sofosbuvir (ALS-220) (Shahid et al., 2014). Sofosbuvir was the first nucleoside/nucleotide inhibitor to be licensed and has high antiviral capacity. Sofosbuvir is also promising due to the genetic barrier to resistance (Kim et al., 2014).
Non-nucleoside inhibitors (NNIs) cause conformational changes of the RNA polymerase by binding to multiple domains on the outside of the active site. The RNA polymerase activity will thereby be inhibited (Shahid et al., 2014, Feeney & Chung, 2014). Dasabuvir is a non-nucleoside inhibitor which is still studied in clinical trials. Combination therapy of Dasasbuvir with other direct-acting antivirals increases the sustained virologic rates to 95% in patients infected with HCV genotype 1 (Gentile et al., 2014). VX-222 and ABT-072, other NNIs, are also under investigation (De Clercq, 2014). VX-222 has been shown to exhibit antiviral activity against genotype 1a, 1b and 2a (Jiang et al., 2014). Lawitz and colleagues studied the effect of ABT-072 against HCV genotype 1 (Lawitz et al., 2013). These NNIs, in combination with other direct-acting antivirals or interferon and/or ribavirin have recently been studied in phase II studies (clinicaltrials.gov, Lawitz et al., 2013).
Unlike in protease inhibitor-based therapy, in NS5B polymerase inhibitor-based therapy resistance to antivirals is not seen very often. NS5B polymerase inhibitors contain thus a high genetic barrier to viral resistance. Nucleoside/nucleotide analogue inhibitors do not show much toxicity (Feeney & Chung, 2014).

Combination therapy and limitations

Most of the clinical trials study the efficacy of DDA therapy in combination with standard of care therapy, which include interferons and ribavirin (Chae et al., 2013). These combination therapies are called triple and quadruple therapies. These therapies are an option for difficult to treat HCV patients and for prior null responders. Not many combination therapies have been licensed yet, indicating that the development of combination therapies is in initial stages (Shahid et al., 2014). An example of a combination therapy is the therapy in which the protease inhibitors Telaprevir and Boceprevir are used in combination with standard of care therapy. This therapy has been licensed in 2011 by the US Food and Drug Administration (Ip et al., 2012). The sustained virologic response has significantly been improved due to this therapy (Kim et al., 2014).

Although these treatment strategies show improved sustained virologic responses, these therapies still have several limitations. First, the prevention of emergence of viral escape and viral breakthrough by the treatment is still dependent on the addition of peg-interferon and ribavirin. Peg-interferon and ribavirin are thus still responsible for the success rate of the treatment (Salam & Akimitsu, 2013, Shahid et al., 2014). Secondly, a main concern of this treatment strategy is that it still causes adverse effects (McHutchinson et al., 2009). Third, in prior null responders triple therapy is less effective and patients contraindicated for peg-interferon and ribavirin cannot be administered with triple therapies (Shahid et al., 2014). Furthermore, Telaprevir and Boceprevir are designed to treat HCV genotype 1, which accounts for 60% of all global infections. Patients infected with HCV genotype 1 did respond the least to current therapy (Salam & Akimitsu, 2013). The approval of these protease inhibitors as therapy is therefore thus a major result for HCV genotype 1 patients. However,

patients infected with other HCV genotypes than genotype 1, accounting for 40% of the infected individuals worldwide, will not respond to this combination therapy.

Direct-acting antivirals are not only studied in combination with peg-interferon and/or ribavirin. Currently, in clinical studies the efficacy of direct-acting antivirals without addition of peg-interferon and/or ribavirin is studied, in order to overcome the limitations of triple and quadruple therapies. Also the adverse effects of interferons and ribavirin could be eliminated (Shahid et al., 2014, Chae et al., 2013). Not only the limitations and adverse effects of triple and quadruple therapies are main concerns in HCV therapy. Also the error-prone replication of HCV is a major issue due to the development of drug resistance. Therefore combinations of two or more direct-acting antivirals without peg-interferon and ribavirin as treatment are under investigation (Ip et al., 2012). These interferon free and ribavirin free therapies may be a good option for patients contraindicated for peg-interferon and/or ribavirin. A combination of direct-acting antivirals as therapy is the combination of NS3 inhibitor Asunaprevir and the NS5A inhibitor Daclatasvir. This combination of therapy achieved a promising sustained virologic rate of 95%. The combination of NS5B nucleoside analogue inhibitor Mericitabine and NS3/4A protease inhibitor Danoprevir also showed a high sustained virologic rate, namely 71%. These results indicate that future therapies with DDAs do not need addition of interferons and/or ribavirin (Chae et al., 2013). Although the combination therapies achieve high sustained virologic rates, drug-drug interactions could still occur and should therefore be monitored very carefully (Delaborde et al., 2014).

Other limitations of antiviral treatment

New direct-acting antiviral treatments are thus developed and are under investigation. In several studies researchers mention that direct-antiviral treatment will be the future treatment of HCV (Chae et al., 2013, Welsch et al., 2012). However, the problems of the anti-viral treatments as previously written, limit the capacity of antiviral treatment and are a main concern. Problems include viral drug resistance and viral drug tolerability, as well as adverse effects of the standard of care therapies and some newly developed DDAs. These problems are not the only concern. Anti-viral treatments have not been shown to prevent reinfection with HCV. Once a patient is cured from HCV infection by anti-viral treatment, the patient has not gained immunity against HCV. Especially in ongoing high-risk populations, including people who use drugs, reinfection occurs frequently. The effectiveness of the anti-viral treatments is thus low in these population groups (Cox & Thomas, 2013).

Another problem of antiviral treatment are the very high costs of these drugs. Annual costs for Peg-interferon/ribavirin treatment per individual are more than US$20.000. Treatment with combinations of newly developed anti-viral drugs for only one individual are US$82.000 per year. These high costs make anti-viral drugs unaffordable in the majority of the world (Verma et al., 2014). Also the just licensed therapy against HCV consisting of interferon, ribavirin and NS3/4A protease inhibitors is even most probably unaffordable and therefore unavailable in certain regions due to the high costs (Zingaretti et al., 2014). To implement the antivirals worldwide, the high costs of the antivirals may be the biggest challenge (Feeney & Chung, 2014).

Due to the failure of effectiveness of antiviral treatment in high-risk populations, HCV infection will never be eradicated. Also the already very high costs of anti-viral treatments, will obviously increase when reinfection occurs since patients need to be treated again. Thus not only the adverse effects and resistance of the drug limit the capacity to treat HCV. Also low effectiveness and the unaffordable costs of anti-viral treatment limit the capacity to treat HCV, especially when reinfection occurs.

Therefore prevention of HCV is enormously favourable, which can possibly be achieved by the production of a HCV vaccine (Zingaretti et al., 2014). An effective vaccine could potentially eradicate HCV infection. According to a calculation made by Massad and colleagues, a 100% efficient selective vaccination responsible for life-long immunity is the most cost-effective strategy in treating HCV. The costs for one vaccination are much lower than individual anti-HCV therapy. Massad et al., even used an overestimation of the costs for a vaccine in their calculation. They also calculated that selective vaccination would not even be necessary since indiscriminate vaccination strategies would be more cost-effective compared to anti-viral treatment (Massad et al., 2009). Also in a study almost ten years ago performed by Krahn and collaborators, it was shown that vaccination of high-risk populations including persons who inject drugs would prevent a large amount of new HCV infection cases and HCV-related deaths. Also costs would be reduced. They concluded that vaccination would be very cost-effective, even in average risk populations (Krahn et al., 2005).

Progress of hepatitis C virus vaccines

Although there is no vaccine for the prevention of HCV available yet, the possibility for an effective vaccine against HCV is supported by the observation that around 25% of the patients infected with acute HCV are able to clear the viral infection spontaneously. This shows that effective immune responses can occur during HCV infection (Zingaretti et al., 2014). Progress has been made in the study of HCV vaccines. Prophylactic as well as therapeutic vaccines are under investigation. Prophylactic vaccines induce the production of neutralizing antibodies by activating the humoral response. Neutralizing antibodies bind the virus particles and block receptor binding and cell entry (Ip et al., 2012). The HCV structural proteins E1 and E2 have been used as targets for neutralizing antibodies. Already in 1994, a potential prophylactic HCV vaccine was tested in chimpanzees that were injected with recombinant HCV envelope proteins, E1 and E2. The animals were protected against infection for a brief period. (Choo et al., 1994). Also other prophylactic vaccines have been tested in chimpanzees since (Forns et al., 2000, Verstrepen et al., 2011). According to clinicaltrials.gov, a phase I study was completed in 2013 in which a prophylactic peptide vaccine derived from HCV E1 and HCV E2 was used for immunization of healthy humans. Currently, even a phase II study is performed to assess the safety, efficacy and immunogenicity of a prophylactic HCV vaccine based on the sequential use of AdCh3NSmut1 (clinicaltrials.gov).
However, vaccines inducing neutralizing antibodies may not completely control HCV infection (Cox & Thomas, 2013). Due to HCV infection, T-cell responses which are needed to clear the infection are reduced in the host, as described by Wedemeyer et al. (Wedemeyer et al., 2002). Therapeutic vaccines activate both the humoral as well as the cellular immune responses. Both neutralizing antibodies as well as virus-specific $CD8^+$ cytotoxic T lymphocytes eliminating virus-infected cells are produced. Multiple sorts of therapeutic vaccines have already been studied, including peptide- or protein-based vaccines, DNA vaccines, viral vector vaccines, recombinant yeast-based vaccines and vaccines based on dendritic cells (Ip et al., 2012). Ip and colleagues investigated the efficacy of a Semliki Forest virus vector expressing all HCV non-structural proteins. This vaccine seemed to be very potential for treatment of HCV since T-cell activity was vigorously induced (Ip et al., 2014). Barnes and colleagues currently study an adenovirus-based vaccine. They show that this vaccine provides protective immunity. This study will be completed in 2016 (Barnes et al., 2012). The development of vaccines against HCV is thus in progress and will hopefully lead to an effective HCV vaccine in the near future.

Discussion

Worldwide, around 200 million individuals are infected with hepatitis C virus and every year 350.000 persons die because of the consequences of HCV. HCV infection is therefore a serious global public health problem (Shahid et al., 2014).
In this essay it has been described that standard of care therapy has limited efficacy and is associated with multiple frequent and serious side effects. Thanks to available information about the molecular biology of the virus, direct-acting treatment against the HCV non-structural proteins NS3, NS4A, NS5A and NS5B have been developed. However, the newly developed treatments also contain several limitations, including low genetic barrier to viral resistance, adverse effects, and drug tolerability by the virus. Not only these limitations are a problem. Antiviral treatment does not induce immunity in patients. Reinfection can therefore occur, especially in high-risk populations including people who inject drugs. Due to the low efficiency of antiviral treatment in these population groups, the virus will never be eradicated by antiviral drugs. Another not insignificant problem of antiviral treatment are the very high costs which are unaffordable in most of the world. Due to these high costs, antivirals cannot be implemented all over the world. Due to reinfection, the unaffordable costs will increase resulting in reduced capacity to treat viral infection. Therefore prevention of HCV is enormously favourable to eradicate HCV infection, which can probably be achieved by the production of a HCV vaccine. Several studies provided calculations about the cost-effectiveness of a potential effective vaccine, showing that vaccination would be the most cost-effective to reduce mortality and morbidity worldwide compared to antiviral treatment

Future perspectives of antiviral treatment
Of course, the development of new direct-acting antivirals is enormously favourable for the treatment of HCV and to reduce the burden of HCV-related diseases. These newly developed drugs could potentially overcome the adverse effects of standard of care therapies and the limitations of current direct-acting antivirals. However, these direct-acting antivirals would never eradicate HCV completely, since antivirals do not immunize individuals against viral infection. Only an effective vaccine could totally eradicate the virus. If such a vaccine would be available in the near future, antiviral treatment would still be needed for a certain period of time to treat already infected patients. But the costs of these antiviral drugs should be reduced to become available for all regions of the world, especially for those which cannot afford the current prices.

Conclusion

In conclusion, at the moment antiviral treatment is needed to treat HCV infected individuals. Due the limitations, high costs and low efficacy of antiviral treatment, the development of an effective HCV vaccine is more favourable to prevent HCV infections, HCV-related diseases and HCV-related deaths.

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