



Antagonizing microRNA-122 and treatment of hepatitis C virus infection.

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Treatment of hepatitis C virus infection

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Summary

Hepatitis C virus infection affects 71 million people worldwide. It is at the origin of a multi-organ disease associating hepatic manifestations, cryoglobulinemic vasculitis and general manifestations linked to chronic inflammation (diabetes, cardio-, reno- or cerebrovascular manifestations, extra-hepatic cancers including non-Hodgkin's lymphoma). The significant morbidity and mortality linked to the hepatitis C virus therefore justify its screening and access to treatments which have increased considerably over the past two decades. Understanding the replicative cycle of the hepatitis C virus has enabled the development of direct HCV-specific antivirals targeting viral proteins (NS3/4A protease, NS5B polymerase and the multifunctional NS5A replication complex). The combination of two to three specific inhibitors often co-formulated in a capsule, without pegylated interferon and most often without ribavirin, allows high antiviral efficacy (more than 97% cure) for a treatment duration of 8 to 12 weeks with satisfactory tolerance. HCV infection is the only chronic viral infection that can be cured and the hepatic or extrahepatic manifestations are mainly reversible. This underlines the importance of strengthening screening and access to care policies in order to achieve the elimination of viral infection C in the short term, in 2030, as expected from the program of the World Health Organization. If this elimination is possible in some countries (Iceland, France, Germany ...), it seems compromised in others where prevention (USA), screening and/or access to care are still insufficient (Sub-Saharan Africa, Russia...).

Keywords: Hepatitis C virus or HCV/Direct antiviral agents or DAAs/HCV protease inhibitors/HCV polymerase inhibitors/NS5A inhibitors/Cirrhosis

1. Introduction

The hepatitis C virus (HCV) was identified in 1988 as an RNA virus responsible for the majority of parenteral-transmitted "non-A, non-B" hepatitis (1). It is the cause of acute hepatitis, spontaneously resolving in one third of cases, and chronic hepatitis with a high rate (70%) of transition to the chronicity, explaining that about 71 million in the world in 2017 still have chronic HCV infection (2). HCV is, at least in industrialized countries, one of the main causes of liver transplantation and hepatocellular carcinoma, which is expected to increase until around 2030 (2-4). The real therapeutic revolution that we have witnessed in the last decade is unique in the speed of development of new treatments (constantly more effective and better tolerated), by the approval of registration agencies for the marketing of molecules sometimes tested without a control group and sometimes on a limited number of patients or by discussing prices with the Economic Committee for Health Products. The uniqueness of the subject is that on the one hand HCV infection is the only chronic viral infection which can be cured (3-4) and that on the other hand the liver has a unique capacity for regeneration and that fibrosis is permanently reshaped by hepatic enzymes, which makes it possible to observe its regression in the absence of hepatic comorbidity, if the necrotico-inflammatory activity is modest or absent (5). This partial (or complete) repair is also observed for extrahepatic manifestations, linked to cryoglobulinemic vasculitis (6) or chronic inflammation (7).

HCV infection is an example of the success of translational research. The effective collaboration between clinicians and researchers enabled the discovery of the virus (1) in 30 years, the development of: a) diagnostic tests (serological and virological) (3-4), b) treatments with Interferon (8), c) predictors of fibrosis progression or of the virological response to interferon, in particular genetic such as the IL28B rs12979860 single nucleotide polymorphism (9), d) specific antivirals inhibiting viral proteins, that allowed the cure of infected subjects (3-4, 10). The challenges today are mainly epidemiological: screening and access to treatment (11) for a frequent infection, a real public health problem.

2. Epidemiology

Between 1990 and 2013, viral hepatitis increased from tenth to seventh rank for the major causes of death, above infection with the human immunodeficiency virus HIV, malaria, or tuberculosis, and are the second leading cause of infectious death worldwide after infections of the upper airways (12).

The prevalence of HCV dropped from around 170 million chronic carriers worldwide in 1999 to 71 million in 2017 (2).

The prevalence's diminution is mainly related to the mortality of infected subjects, but also to the (still minor) improvement of access to treatment, and to the reduction of nosocomial contamination. Currently, 1% of the global population is infected with HCV, with more than 1.7 million new cases in 2015, principally inked to blood transmission of drug use in developed countries, and to the economies and the hemovigilance deficit in developing countries. About 2.3 million people have HIV and HCV co-infection (2). There is a large disproportion in the prevalence of HCV around the world; Egypt (because of delivery bilharzia treatment without sterilized disposable material) and Mongolia (because of the lack of hemovigilance) are the most exposed countries, with 15% of infected patients among the general population. West and Central Africa states are also massively affected with around 5 to 8% of the population, due to in-hospital contamination but also to some folk or tribal habits such as scarification, circumcisions, or excisions (2). In Europe and North America, hepatitis C prevalence is less than 1%, with a significant decline in the past 20 years. In France, the prevalence diminished from 1.2% in 1996 to 0.8% in 2011 and is presumably 0.40 % at most in 2020.

In 2015, there were around 1.2 million of deaths linked to chronic viral (HBV and HCV) infections, two-thirds from cirrhosis and one-third from hepatocellular carcinoma, with a raise of 22% since 2000 (2). Despite the HBV-related mortality is almost the double (even though an available vaccine

for more than 30 years), 400 000 subjects die every year from chronic HCV infection (2). Those data emphasize the requirement for effective HCV screening.

3. Diagnosis of viral infection C

The story of HCV is very singular since it is a virus that was discovered late (1), but in 30 years we have managed to isolate the virus, and the collaboration between basic and clinical research has allowed the development of reliable diagnostic tests (3,4) , the discovery of effective treatment (8,10), this allowed the healing of infected subjects and to evoke the elimination (2). This program, supported by the World Health Organization (WHO) is expected to reduce new infections by 30% in 2020 and 90% in 2030 and to decrease mortality from hepatitis by 10 and 65%, respectively.

It was in 1998 that Michael Houghton's team manage to isolate complementary DNA from the blood of a patient infected with non-A non-B virus, consenting the isolation of HCV RNA (1) and the rapid development of diagnostic tests (3 -4). In parallel, ELISA tests and immunoassays (now discontinued) have been developed. The sensitivity and specificity of serological tests has been increasing with today an excellent diagnostic capacity. The only limit is the period of seroconversion of a few weeks compared to contamination, unlike viremia, which is detectable within 4 days of encountering the virus. The isolation of HCV RNA (by RT-PCR) allowed the sequencing of the virus, the definition of different genotypes (numbered from 1 to 7) and subtypes (a, b, c...), the development of resistance tests and quantitative viremia. Before the identification of HCV, standard Interferon therapy for non-A non-B hepatitis had started in the late 1980s (8). It had been shown that 3 to 5 million units of Interferon 5 days per week or every 2 days were enabled in half of the cases a biological normalization, associated with viral eradication, defined by an undetectable HCV RNA in 24 weeks after the end of treatment, in half of the cases when virological tests were available. In parallel with virological tests, serological tests have been developed to identify the capsid antigen of the virus, having a sensitivity and specificity comparable to HCV RNA, but which have not known the expected developments, although it may be a cheaper test and as effective as viral load. The development of

serological tests allows the identification of infected subjects: infection by HCV is characterized by the presence of anti-HCV antibodies but active infection is only strictly defined by the detection of HCV RNA by RT-PCR in the serum (Figure 1). Thus, a patient with anti-HCV antibodies and an undetectable viral C RNA is an exposed subject but cured of his infection (3-4). Serological tests also enabled mass screening, in particular of blood donors. After the identification of markers of viral B infection (anti-HBc antibody, HBs antigen leading to the exclusion of blood donation), the identification of anti-HCV antibodies made it possible to collapse the risks of viral C transfusion contamination which today are only around 1 in 700,000 to 1 million corresponding to occult infections thanks to viral genomic diagnosis. The identification of HCV has transformed patient care and the prevention of nosocomial risk in addition to risk reduction measures, particularly in drugs users.

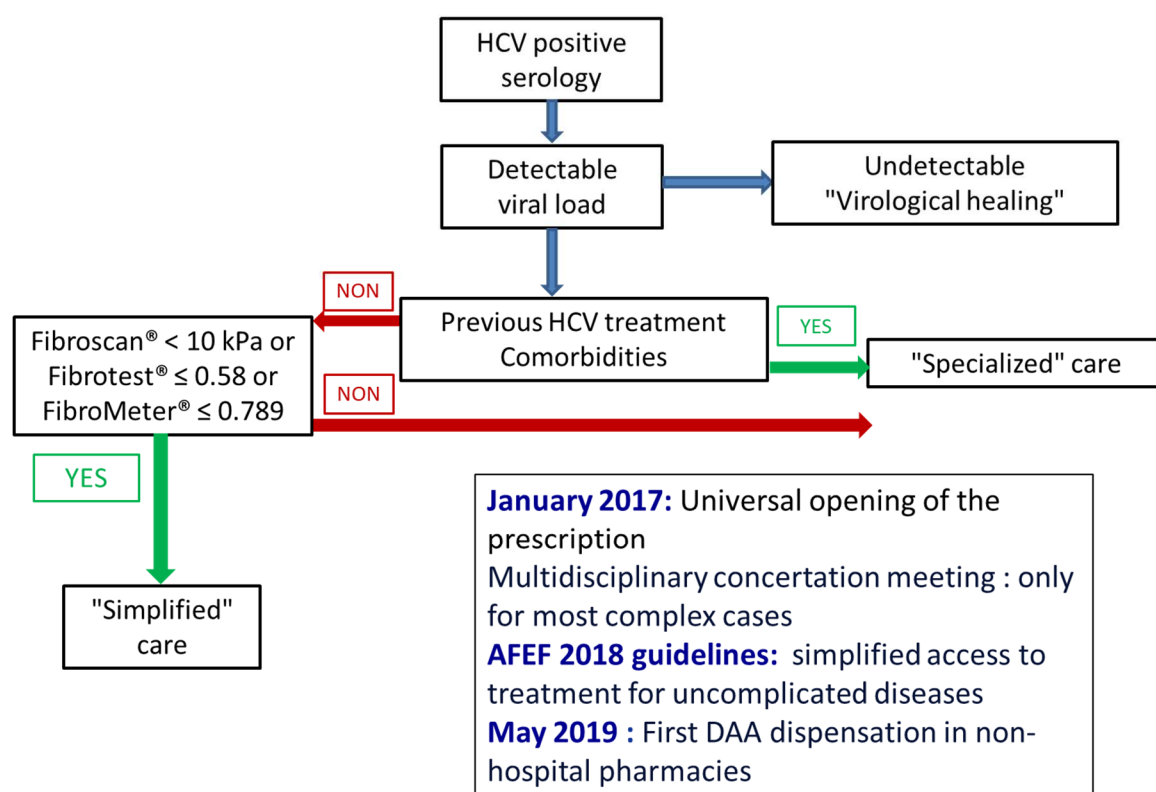


Figure 1. Diagnostic and therapeutic management of hepatitis C virus infection. A simplified pathway is offered to patients without hepatic comorbidity, while for the others, specialized monitoring and management are recommended.

4. Why to treat Hepatitis C virus infection

4.1. Chronic HCV infection, a systemic disease

HCV is a hepatotropic and lymphotropic virus (Figure 2). Hepatotropism is responsible of chronic hepatitis, which can progress to cirrhosis and hepatocellular carcinoma (3-4). Viral infection C is a systemic disease caused by the direct toxicity of the virus and by immune-mediated mechanism (7): chronic hepatitis is related to the action cytotoxic T lymphocytes recognizing the viral antigens expressed on the surface of the hepatocytes. Lymphotropism, characterized by viral C multiplication inside B lymphocytes or by their chronic stimulation by viral infection, explains the detection of cryoglobulinemia in approximately 50% of HCV infected patients (6). HCV associated cryoglobulin is type II combining a monoclonal IgM contingent and a polyclonal IgG contingent. It is a proteic complex combining HCV, antiviral antibodies and rheumatoid factor which can deposit in the walls of small and medium-sized vessels and then cause cryoglobulinemic vasculitis (13). Cryoglobulinemic vasculitis can be responsible for purpura or necrotizing acrodermatitis, membranoproliferative glomerulonephritis, peripheral neuropathies and polyarthrititis of small joints. At most, B lymphocyte infection or chronic antigenic stimulation of B lymphocytes can be associated with clonal selection responsible for lymphoma, mainly non-Hodgkin B lymphoma, splenic lymphoma type or more diffuse lymphomas (7, 14).

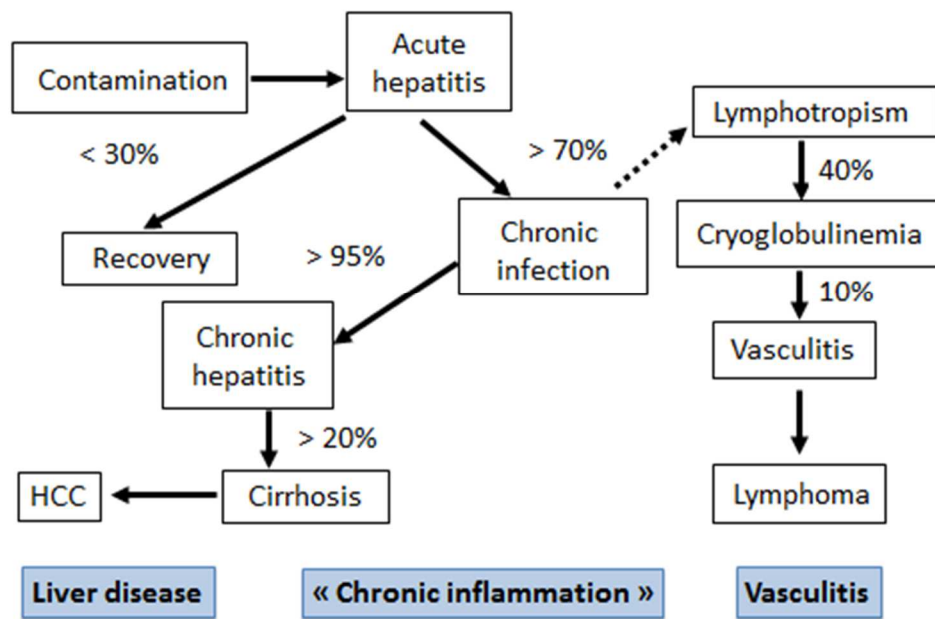


Figure 2. Natural history of Hepatitis C infection associating, in the absence of spontaneous recovery in a third of cases in the acute stage of infection, hepatotropism, lymphotropism and chronic inflammation with their hepatic and extrahepatic consequences.

HCV chronic infection (75% of the infected patients) is associated with chronic inflammation, which can cause extrahepatic manifestations associating neurocognitive disorders, insulin resistance and diabetes, cardio-, cerebro- or renovascular diseases and extrahepatic cancers (6-7). These hepatic and extrahepatic damages account for a ten- and two-times higher hepatic and extrahepatic mortality respectively in anti-HCV positive patients with detectable viral C RNA compared to anti-HCV positive patients without detectable HCV RNA or in patients who have never encountered HCV (anti-HCV negative) (15). When viral infection C is confirmed (HCV RNA detectable by RT-PCR), it is important to assess the clinical, hepatic and systemic consequences. The assessment of liver disease, that was formerly carried out by liver biopsy on the basis of semi-quantitative scores like Metavir (16), has been revolutionized by the non-invasive tests for liver fibrosis including biological (Fibrometer , Fib4, Fibrotest, , Hepascore, or Apri) (17-20) or morphological tests, in particular evaluation of liver stiffness by elastometry (Fibroscan, Supersonic SSI) (21-22) (Figure 1).

Correspondences between the values of these tests allow an evaluation of fibrosis (F) on a conventional scale from 0 to 4 where F0 and F1 which is equivalent to minimal fibrosis, F2 to medium, F3 extensive or F4 cirrhotic, that justifies semi-annual ultrasound monitoring of patients due to the risks of hepatocellular carcinoma.

4.2. HCV infection, the only chronic viral infection that you can cure

HCV virus is a single-stranded RNA molecule with positive polarity of approximately 9,600 nucleotides. The genome codes for a single 3000 amino acid polyprotein which is split by a protease into structural and non-structural proteins (23-24). NS5A protein is also called replication complex and it is crucial for replication process and for assembling the virion; NS5B and NS3/4 protease are the most important authors for viral replication (Figure 3). Direct antiviral drugs have allowed, since 2005, the cure of chronic HCV infection through their selective action on these non-structural proteins (10, 25). Unlike the hepatitis B virus or HIV that integrate their genome into the host cell, HCV replication is entirely cytoplasmic and this has allowed the cure of hepatitis C which corresponds to a negative viral load 12 weeks after the end of the treatment (prolonged viral response) (3-4). Since, as mentioned above, there are no virus reservoirs, this virological healing correspond to the absence of the virus in the peripheral monocytes and hepatocytes (26). Consequently, unlike HBV, the organs of a healed person can be transplanted without the need to undertake antiviral treatment in the recipient, despite immunosuppressive treatment (27).

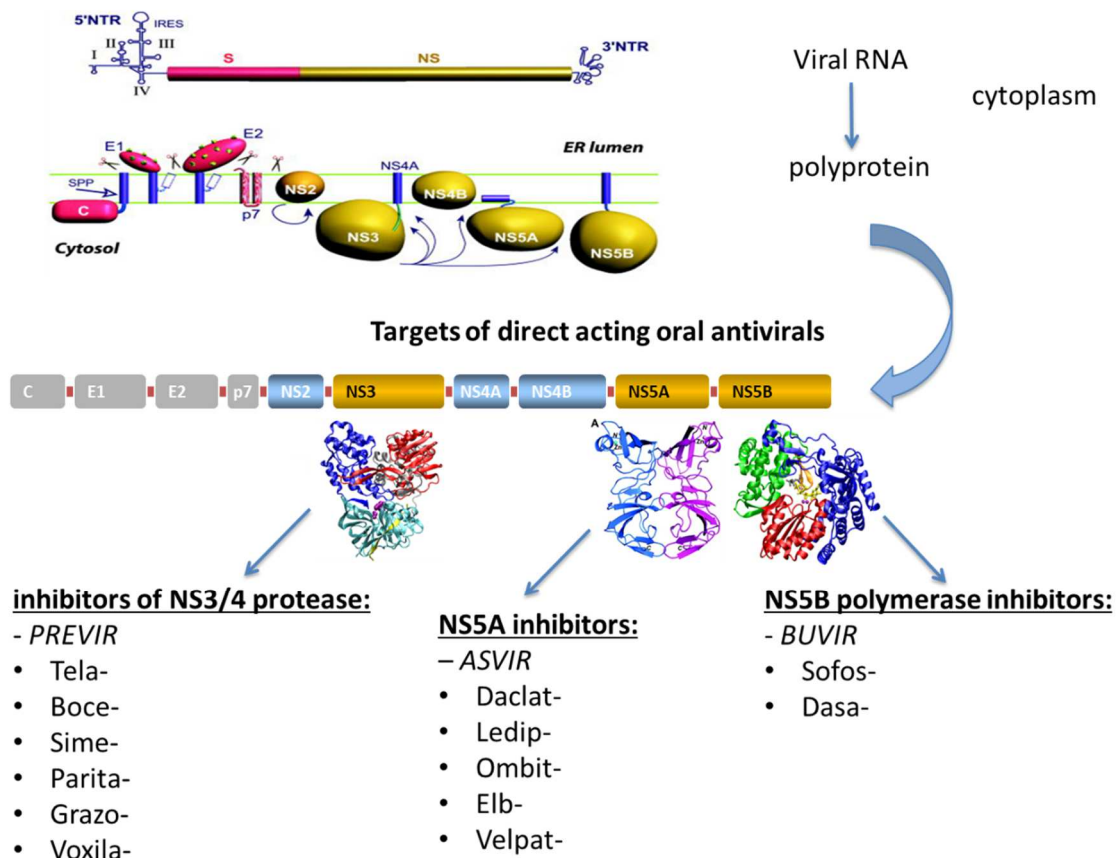


Figure 3. Summary of the biology of the hepatitis C virus: HCV RNA replication is dependent on RNA polymerase. HCV RNA is translated into a polyprotein split into structural (envelope and capsid) and non-structural proteins, some of which are the specific targets of oral antivirals, inhibitors of the NS3/4 protease, of the NS5A replication complex or of the NS5B polymerase.

4.3. Clinical benefits of virologic recovery with reversibility of clinical and biological manifestations

Virological healing is associated with an amelioration of hepatic and extra-hepatic manifestations (3-4, 6, 13), allowing the reduction of liver and non liver- related mortality (15). For example, in the prospective cohort of compensated cirrhotic patients in the CIRVIR cohort from the ANRS-INSERM, a reduction in the risks of occurrence of hepatocellular carcinoma has been observed at 3 and 5 years in cured patients (13.6 versus 1.9 and 21, 8 versus 3.3% incidence of CHC) (28). It is interesting to note that in patients who have recovered from viral infection C, HCC is only observed in the case of metabolic syndrome or hepatic comorbidity (overweight, diabetes, excessive alcohol intake) (29). Virological recovery allows, in addition to a reduction in HCC observed in cirrhotic and non-cirrhotic

patients, an improvement in the life expectancy of patients with a reduction in hepatic mortality but also in overall mortality (30). The reduction in liver mortality is related to the ability to remodel fibrosis with the possibility of reversibility of cirrhosis which has been reported to reduce the risk of mortality (5, 31). In parallel with a significant reduction in the risks of decompensation of cirrhosis, a reduction of the same magnitude in the risks of bacterial infections has been proven and, more demonstratively, a reduction in vascular risks (9.1 to 2.3% at 3 years and 12.3 versus 3.5% at 5 years in cirrhotic patients cured compared to non-cured patients) (29).

Moreover it has been demonstrated that cirrhosis is associated with an increased risk of carotid arteriosclerosis (32) and that intimal carotid thickness decrease after oral antiviral therapy (33).

4.4. HCV infection is contagious

Beyond the hepatic and extra-hepatic risks linked to HCV, the infection exposes to a risk of contamination in communities with risk behaviors, including mainly men who have sex with men (MSM), intravenous drug users (IDU), prisoners, migrants. Very early, and even in countries that have proposed a treatment prioritization policy such as France (oral antiviral treatments were authorized from December 2013 in the most severe patients on the hepatic level, then in those with significant fibrosis F3-4 or extrahepatic manifestations between 2014 and 2015, then F2 patients in 2016 before the opening of the prescription for all patients in January 2017), patients at high risk of contagiousness, such as those infected with HIV , could be treated. Systematic treatment of chronically infected patients from high-risk communities or those with acute hepatitis has been shown to significantly reduce the risk of contamination or reinfection (34). The healing obtained by antiviral treatments thus allows individual benefits as well as collective benefits.

5. How to treat?

5.1. Direct-acting antiviral (DAA) treatments

Interferon (IFN) has been used for more than 20 years for chronic HCV treatment, due to its antiviral and immunostimulatory properties (8).

Its pegylation (PEG-IFN) allowed weekly subcutaneous injections rather than at least triweekly and the association of a nucleoside analogue, Ribavirin (RBV), made it possible to significantly increase the effectiveness of these treatments (35- 36) (Figure 4). The limitations of this combination were the poor clinical tolerance (acute dysimmune pathologies, flu-like syndrome, neurocognitive disorders) and biological (neutropenia and thrombocytopenia for IFN, hemolytic anemia for RBV). The cure rate was at best 50% with a treatment during 48-weeks for genotypes 1 and 4 and 24 weeks for genotypes 2 and 3. A large number of predictive factors of non-response were identified such as extensive fibrosis, overweight, genotypes 1, associated HIV infection or insulin resistance, and the absence of IL28B rs12979860 CC favorable genotype (9, 35-38).

The availability of specific inhibitors of direct oral viral or antiviral proteins (DAA) thus constituted a real break in the therapeutic landscape. The first oral antiviral was BILN 2061, a protease inhibitor in 2003 whose development was halted due to cardiac steatosis in massively exposed monkeys (39). The first protease inhibitors to have had a marketing authorization between 2011 and 2014, Telaprevir and Boceprevir, were combined with PEG-IFN and RBV but allowed a reduction of the length of treatment and cured approximately three quarters of patients (40). The development of the first polymerase inhibitor, sofosbuvir, and the first in-class NS5A inhibitor, daclatasvir, transformed the prognosis and tolerance of treatments because approximately 95% of patients receiving them, first with PEG-IFN/ RBV and then in combination orally, were cured (41) (Figure 4). As they developed rapidly, combinations of 2 or 3 DAAs, co-formulated or not, were used and made it possible to cure between 95 and 98% of the patients treated, with comparable results regardless of the combination (not face-to-face comparison study of the different associations) and with very good tolerance. The combinations other than sofosbuvir/ daclatasvir (41) were not pan-genotypic; for example, the combination grazoprevir/ elbasvir was only recommended for genotypes 1 and 4 (42).

In approximately 3 to 5% of cases (25), oral combinations are associated with failure reflecting either non-adherence to treatment in patients with chaotic pathways or virological failure reflecting virological resistance detected during (virological breakthrough marked by an increase in the viral load under treatment) or after treatment (relapse marked by a reappearance of a detectable viral load after stopping treatment). Numerous resistance mutations, from the NS3 regions and especially NS5, have been identified, associated with virological failure. It is not recommended to identify them before a first treatment because their presence has little effect on the general effectiveness of primary treatments (3-4, 25). In case of virological failure, it is advisable to do a resistance genotyping which will rarely modify the therapeutic choice: retreatment, possibly adapted according to resistance genotyping, allows more than 90% virological cure (see below).

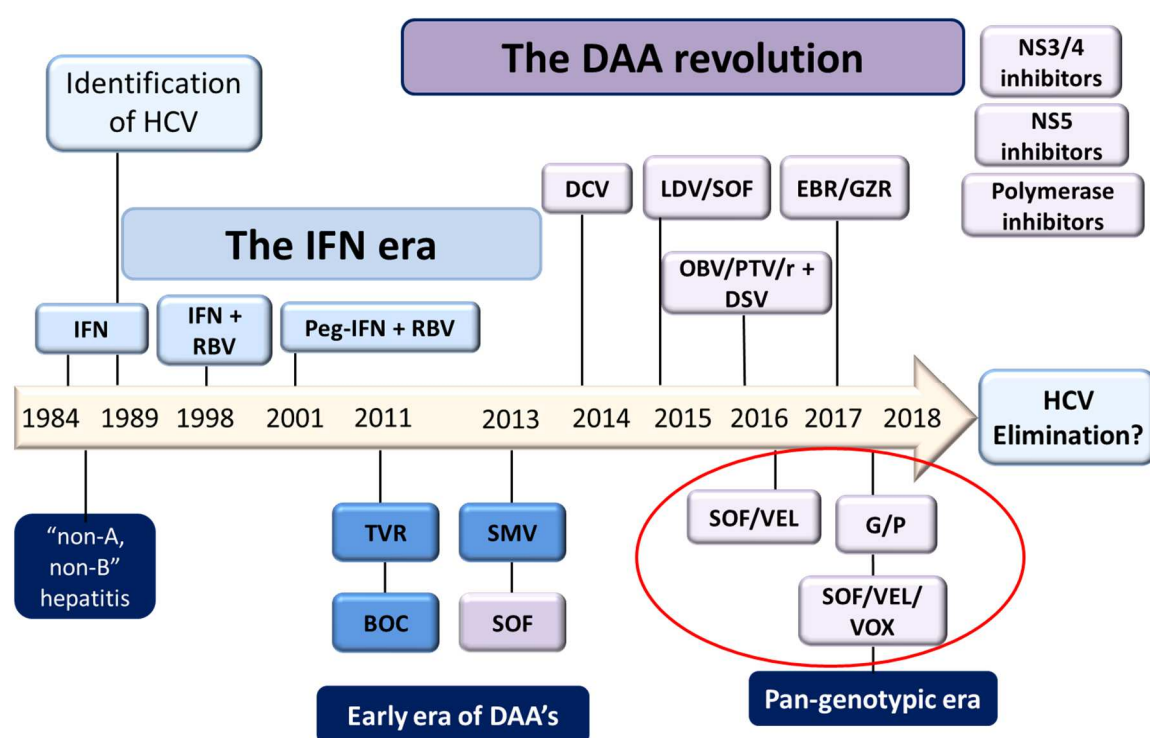


Figure 4. Direct acting oral antiviral agents available in 2020 that cure approximately 98% of patients treated for 8 to 12 weeks mainly. IFN = Interferon; PEG-IFN = Pegylated interferon; RBV = Ribavirin; TVR = Telaprevir; BOC = Boceprevir; SIM = Simeprevir; SOF = Sofosbuvir; DCV = Daclatasvir; LDV = Ledipasvir; VEL = Velpatasvir; VOX = Voxilaprevir; G / P = Glecaprvir / Pibrentasvir. The direct antivirals in –previr are protease inhibitors NS3/4, in –buvir NS5B polymerase inhibitors and in –asvir NS5A inhibitors.

5.2. Therapeutic recommendations in 2020

In 2020, the combination of 2 to 3 DAAs cures approximately 98% of patients for treatment periods of 8 to 12 weeks with 1 to 3 capsules per day; the antiviral power of these pangenotypic treatments has canceled out the factors of poor response. A “simplified route” is possible with general practitioners for patients without hepatic comorbidity and without extensive hepatic fibrosis (Figure 1). The therapeutic recommendations (3-4) are: 1. to treat all HCV positive patients by prioritizing those with advanced hepatic fibrosis, vasculitis, or risks of rapid progression of fibrosis such as hepatic comorbidities or previous transplantation; 2. ban protease inhibitors, potentially hepatotoxic, in decompensated cirrhosis (even if it has been rewarded) (40,43); 3. in the latter case, use sofosbuvir combined with velpatasvir and ribavirin (12 weeks).

The advantage of HCV treatment is surely individual and also collective since it allows the reduction the risks of infection and re-infection in groups at risk such as MSM or IDU (34). The only limits that can be completely circumvented today in treatments are drug interactions (electronic sites allow easy risk management) (44), therapeutic compliance which justifies therapeutic education for difficult populations, and the rare side effects, mainly observed for the most severe patients (45). To summarize, HCV treatment's history is: 1. multiple combinations with high pangenotypic efficacy, easy to use (one to three capsules per day) for 8 weeks - combination glecaprevir / pibentrasvir (Maviret) (46- 49) - or 12 weeks - sofosbuvir / velpatasvir combination without (Epclusa) (50) or with voxilaprevir, (Vosevi, even Sofosbuvir and Maviret, often reserved for failures of the first treatments with DAA) (51-52) and with good tolerance and little safety issues (45).

6. World Health Organization (WHO) elimination policy

Virological healing induces a collective and individual benefit with stabilization or improvement of clinical and biological manifestations. On these evidences is based the elimination policy proposed, from 2017, by the World Health Organization.

With the efficacy of pangenotypic oral antivirals, elimination (and not eradication in the absence of an effective vaccine) is possible (2). From the availability of effective drugs, only the 5% of the infected patients worldwide has been treated, due to the low diffusion of screening and the difficult access to treatment, as well as the high cost of drugs (11). Of the 5 million patients treated and cured, 3 million were treated with cheaper generic drugs. The universal treatment and the simplification of therapeutic strategies should make the prevision of elimination possible (53-54) subject to: 1. improving screening policies by rapid diagnostic tests (55-56) or even Point of Care tests (POC) (57); 2. opening, beyond hospital structures and specialized in hepato-gastroenterology, infectiology or internal medicine, access to prescription for all doctors, which was done in France in May 2019; 3. re-localizing diagnosis (identification of subjects with active viral infection C (detectable HCV RNA)) and initiation of treatment (test and treat) (54) in the various care structures, sexual health, the care structures of different communities at risk, in prisons, in addictology centers, in maternities, in migrant care centers, in psychiatry services: this will reduce the risk of primary infection and re-infection (58). The availability of low-cost, easy-to-use treatments is expected to lead to successful elimination, pending the eventual availability of a preventive vaccine.

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