White Paper Report

Why Hepatitis C Vaccines Will Increase Prevention, Clinical Savings And Cost Effectiveness Together With Supporting DAA Treatments In Toolkit For Attaining Sustained Elimination In The 2030 Framework For Viral Hepatitis Goals

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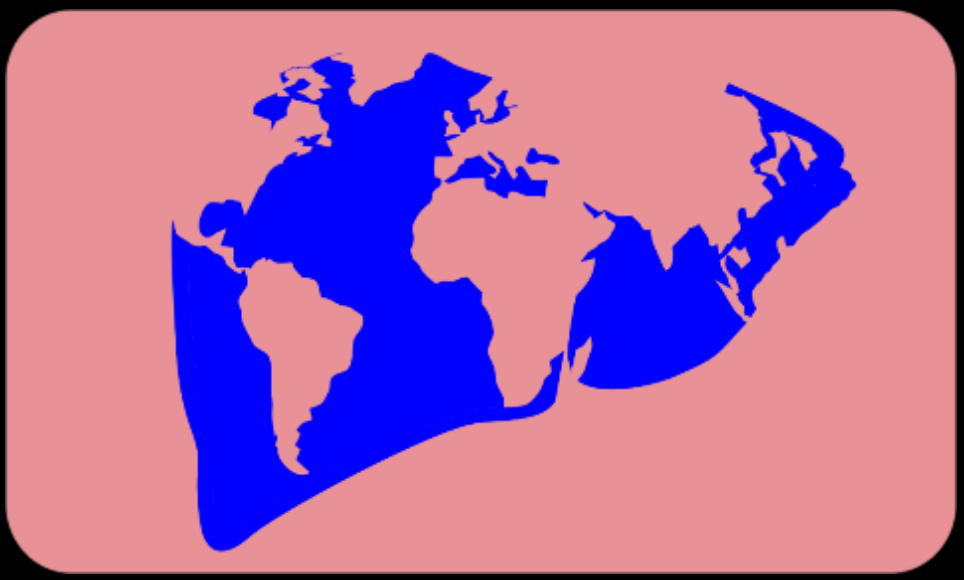

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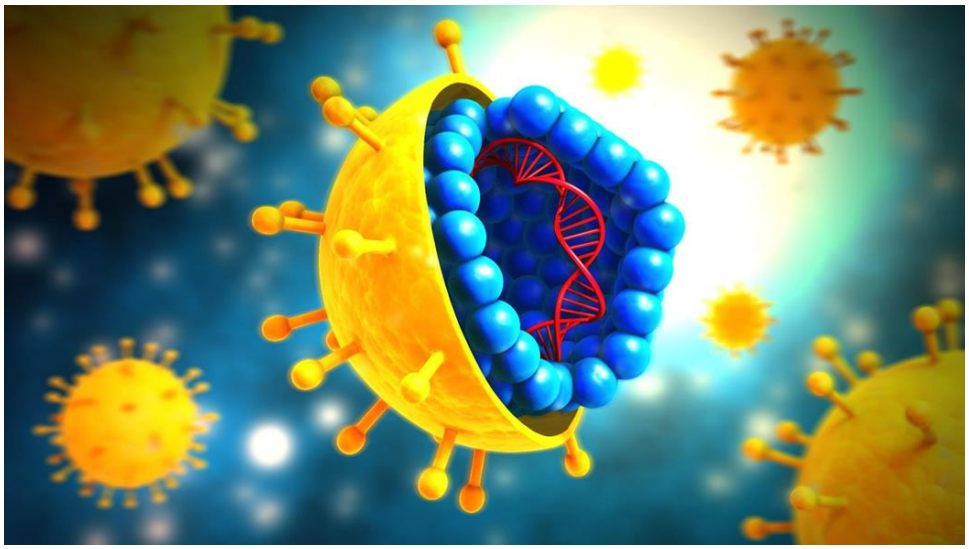


1. Introduction

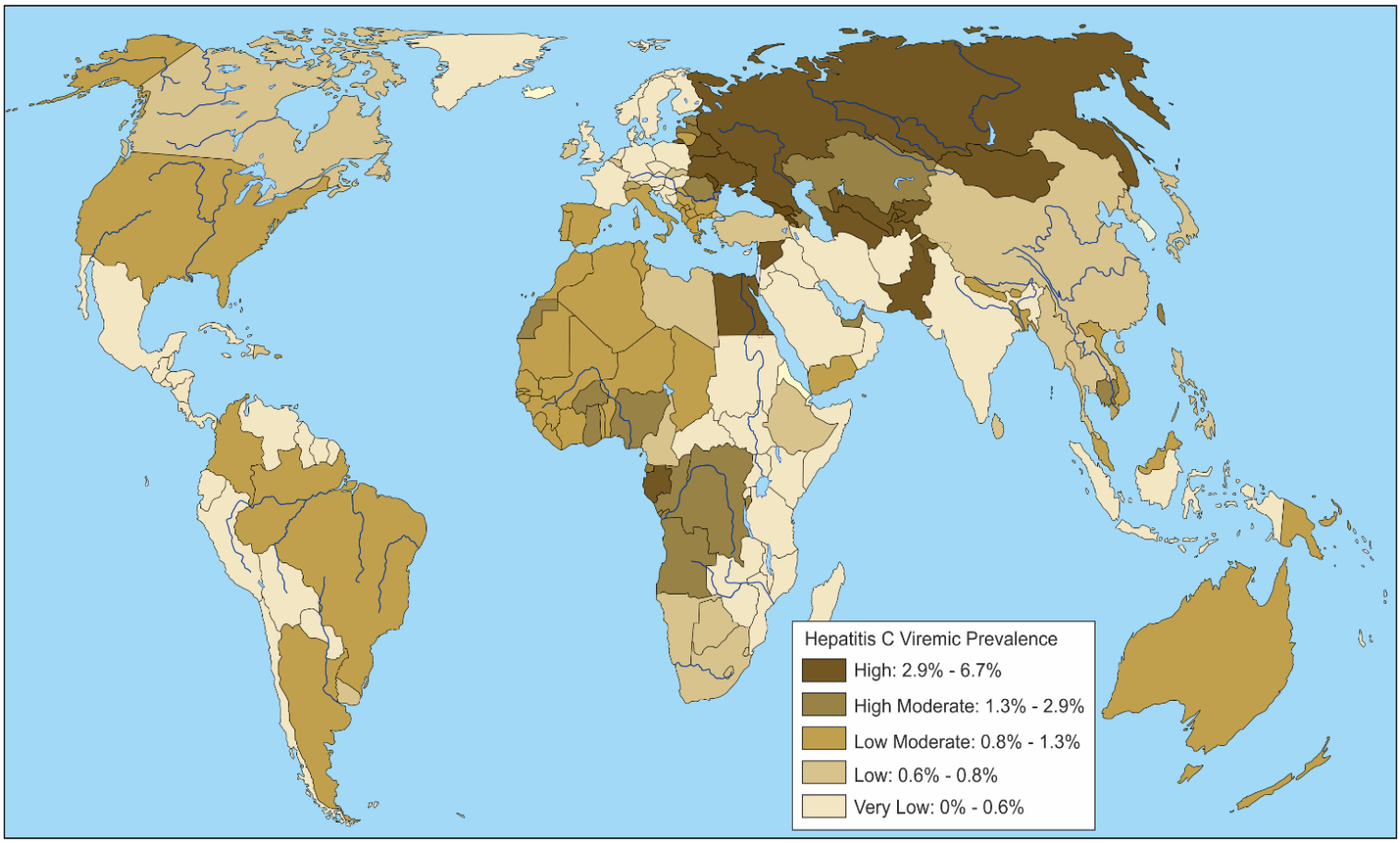
1.1 The burden of Hepatitis C: A geostatic analysis

Hepatitis C virus (HCV), discovered in 1989, represents an important health burden. Hepatitis C virus is a spherical, enveloped, positive-strand RNA virus that belongs to the Flaviviridae family, genus *Hepacivirus*. Seven distinct HCV genotypes and 67 subtypes have been identified [1], the distribution of which varies geographically worldwide.

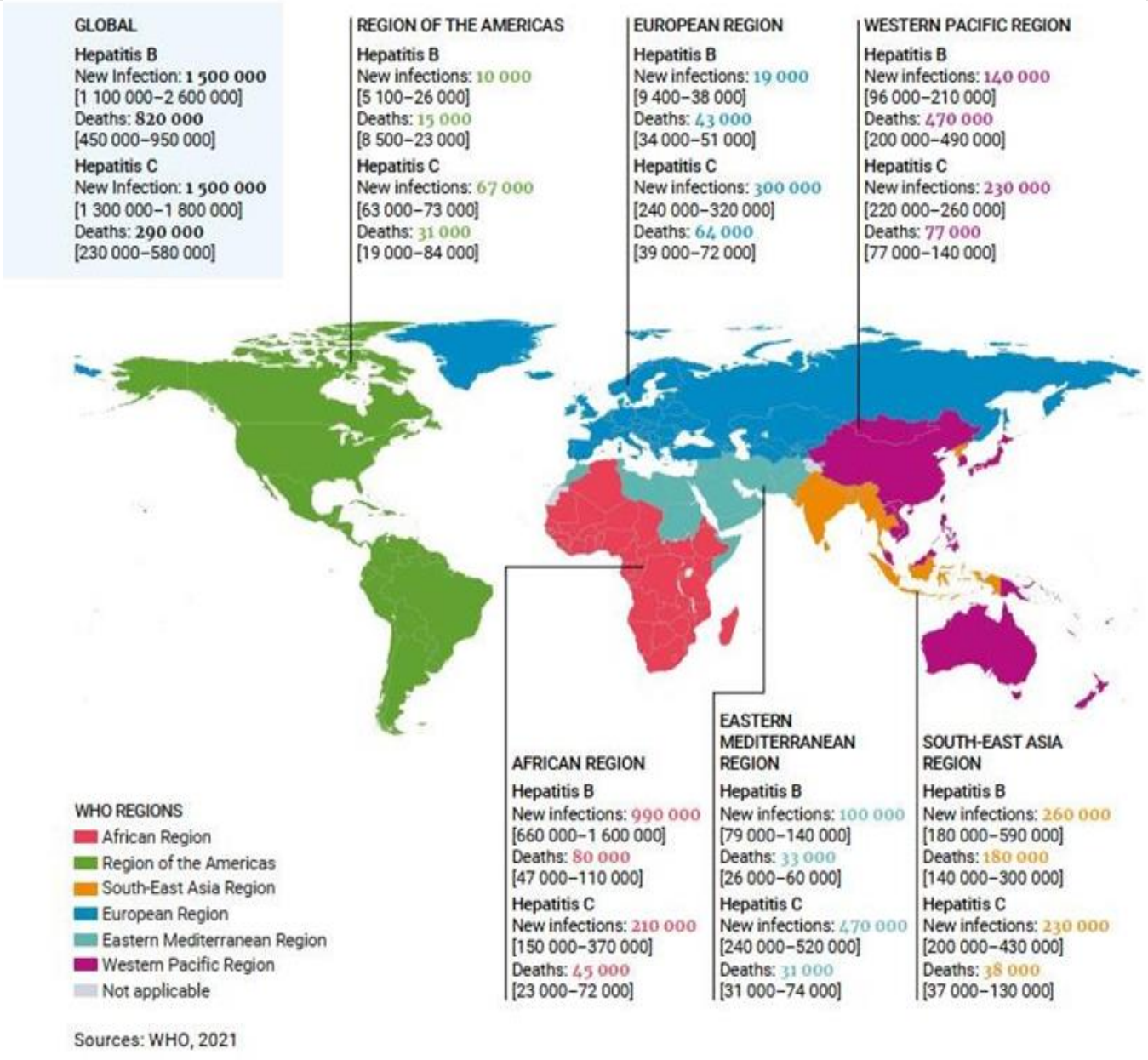
Infections with HCV cause both acute as well as chronic liver disease in 60%-80% of the cases and chronicity is reported to be associated with the development of cirrhosis (15%-30%) and hepatocellular carcinoma (HCC) [2]. Liver damage caused by this infection makes it one of the most frequent indications for liver transplantation worldwide [3,4]. Patients can be fully cured from chronic hepatitis C virus (HCV) infection thanks to antiviral therapy before requiring a liver transplant.

Interestingly, recent studies showed that reinvigoration of certain immune traits can be seen, but many features of immune exhaustion continue over time after viral elimination. Furthermore, it was demonstrated that HCV infection alters the position of histone modifications, thereby inducing an epigenetic signature that persists following the cure with antivirals and these changes can be reverted by specific drugs [5,6]. More detailed studies on exhausted HCV-specific CD4+, CD8+ T cells in chronic HCV and studies on the epigenetic imprint of immune cells after successful treatment are warranted.

Even if the quality of epidemiologic data and prevalence estimates differ widely across countries and within regions, recent global estimates in term of a “global visualization map,” presented by *Lancet Gastroenterol Hepatol. (*2017) and reported by CDC [7,8] indicated that the viremic prevalence of HCV infection is <1.0% in most developed countries, including the United States. The prevalence was considerably higher in some countries in Eastern Europe (3.3% in Russia, 2.2% in Latvia) and certain countries in Africa (6.3% in Egypt, 7.0% in Gabon), the Middle East (3.0% in Syria), the South Caucasus and Central Asia (4.2% in Georgia, 4.3% in Uzbekistan) and southern and eastern Asia (3.8% in Pakistan, 6.4% in Mongolia, 2.1% in Taiwan). We report the data showing the 2014- HCV viremic prevalence **Fig.1A.**


Hepatitis C Viremic Prevalence
High: 2.9% - 6.7%
High Moderate: 1.3% - 2.9%
Low Moderate: 0.8% - 1.3%
Low: 0.6% - 0.8%
Very Low: 0% - 0.6%

(A)



(B)

Fig 1-Viremic prevalence of Hepatitis C. Figures are readapted from [8,9]. Viremic prevalence of HCV infection was reported by CDC in a global map in 2017 (A). Hepatitis B and C new infections and deaths are reported in the WHO regions. Data were estimated in 2019 (B).

According to 2019 data (as updated by the World Health Organization - WHO), 58 million people live with chronic hepatitis C [9]. **Fig.1B** presents Hepatitis B and C new infections and mortality by WHO region; new infections and deaths were estimated around 1 500 000 and 290 000, respectively. The figure also reports global data relative to Hepatitis B, another potentially life-threatening liver infection.Updated WHO Guidance on HCV infection regarding HCV testing and treatment was released on the 24th of June 2022 during a joint WHO-EASL-CDC symposium at the EASL International Liver Congress 2022 in London.

The lack of accurate and timely data hinders our understanding of the disease burden, prompting a cautious interpretation of the recent 2022 CDC surveillance report [10].The need to collect new data after the Covid-19 pandemic is clear since a dearth of accurate and timely data impedes our ability to understand the disease burden : identification of trends in sociodemographic characteristics, risk factors, focusing resources in geographic areas and communities as well as settings where they can be most effective are pivotal objects of studies [11].

Hepatitis C is a bloodborne pathogen, which means that it spreads through blood contact. The higher risk populations in the United States and in most developed countries include people who inject drugs and men who have sex with men, particularly those infected with HIV or those who are taking pre-exposure prophylaxis against HIV. In developing countries, injection drug use also can be an important risk factor for HCV transmission, but the most common modes of transmission are healthcare associated with poor infection-control practices [12]. Occupational exposure in health care fields (medical, dental, or laboratory) to human blood may place a health care worker (HCW) at risk of hepatitis B virus (HBV), HCV, or HIV infection. Moreover, there are an estimated 10 million prisoners worldwide (over 2 million people incarcerated in the USA alone) and this population has shown an extremely high prevalence of chronic HCV infection: less than one percent of eligible prisoners living with HCV are currently treated while incarcerated [13].

Travelers' risk for contracting HCV is generally low. The following activities, as reported by WHO, can result in significant blood exposure: "*receiving blood transfusions that have not been screened for HCV,having medical or dental procedures, activities such as acupuncture, tattooing, or injection drug use in which equipment has not been adequately sterilized or disinfected or in which contaminated equipment is reused*" [9].

In 2016, the WHO approved the Global Strategy to eliminate HCV infection by 2030 [14]. To achieve this goal, the WHO plans to attain "a 90% reduction in new cases of chronic hepatitis C, a 65% reduction in hepatitis C deaths, and treatment of 80% of eligible people with chronic hepatitis C infections". Since 2015 the number of the population who received treatment for hepatitis C increased to 9.4 million (9-fold). The HCV eradication can be achieved only when synergistic prevention and treatment activities are implemented.

Models of prevention have been extensively described. For instance, the control of blood donors and the prevention of transmission of HCV among persons who inject drugs, as well as models of treatment that involve the elimination of HCV through test and treat strategies which use direct acting antivirals (DAAs) with high efficacy and excellent safety. Most patients can be cured with genotypes 1, 2, and 4 in 12 weeks, yet many genotype 1 patients, and all genotype 3 patients require 24 weeks of treatment [15].
However, there are some challenges to manage for a full and complete eradication. Below, challenges discussed in literature studies [2,13,14,15,16,17,18] are reported:

1. Though improvements in screening, treatment, and linkage to care for at-risk populations have been achieved, many people with Chronic Hepatitis C (CHC) have not been tested or treated in most countries since acute HCV usually goes undiagnosed because it is often asymptomatic.

2. HCV management within the prison setting depends on screening, clinical and laboratory assessment, specialist assessment, treatment access and confirmation of cure. At each step, organizational and financial barriers have habitually limited the number of prison-based treatment.

3. It is important to assess the latest estimates of prevalence, especially in high-risk groups, for cost of illness, cost-effectiveness, and budget impact studies. Interestingly, the Clinton Health Access Initiative (CHAI) and The Hepatitis Fund published two Memoranda of Understanding to dramatically lower the price of WHO prequalified hepatitis B (HBV) and C drugs in low- and middle-income countries. HBV/HCV co-infection is a severe liver disease and has an increased risk for liver cancer. Successful use of the hepatitis B vaccine [19], as a model can demonstrate what can be achieved when an efficient vaccine becomes approved and deployed.

4. The chronic nature of HCV, often in marginalized communities, and in combination with the stigma associated with the disease, has left it an often-neglected disease in comparison to the acute nature of COVID-19, which has focused general efforts to tackle SARS-CoV-2.

5. HCV infection is considered a unique human immunological model for the study of T cell immunity. Virus specific T cells play a central role in spontaneous elimination of acute HCV infection. T-cells becomes functionally exhausted during HCV chronic infection and cure by DAA's may not lead to a complete reversion of T cell exhaustion [20,21].

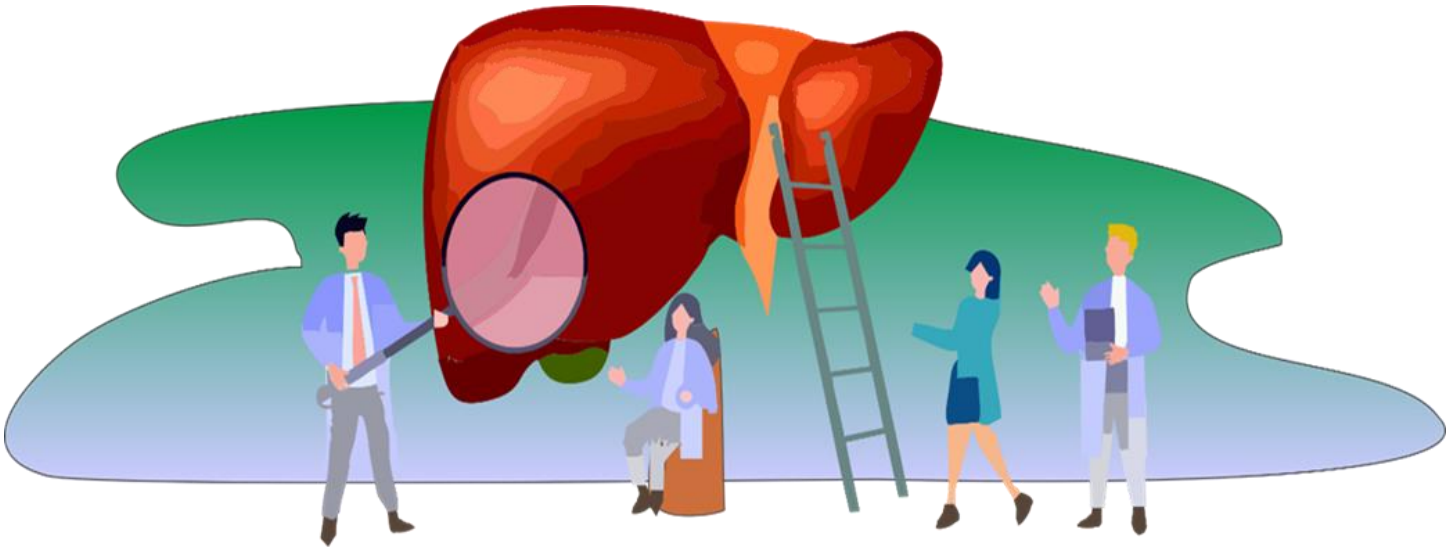
6. The virus's genetic variability has been the object of research studies. The virus diversifies into numerous subtypes, distinct genotypes, and variants (quasi species). This high intrinsic mutational capacity of HCV allows the virus to generate escape mutations which can lead to drug-resistance. Some drug-resistant variants have been isolated and they are the result of different selective pressure [22]. Further studies are necessary for understanding the phenomenon.

7. The recent 2023-CDC report [23] has presented an "HCV clearance cascade" with data for approximately 1.7 million persons in the USA with evidence of a history of HCV infection despite the availability of DAA.

8. The development of an effective prophylactic or therapeutic vaccine becomes necessary to achieve global epidemic control of this virus. Vaccines are needed for long term sustainability of elimination and for maintaining it. However, an efficient vaccine is still missing. Generation of one or more HCV vaccine platforms has generally relied on inducing potent and broad antibody neutralization. The development of effective HCV vaccines was a challenge because of a lack of a robust animal model,

HCV strict species tropism, HCV diversity which reflect differences in the structure of the virus and variables that affected the host [24].

In this work, we discuss the burden of undiagnosed hepatitis C in terms of diagnosis and treatment costs to highlight the importance of investing resources in testing, treatments and in effective prophylactic or therapeutic vaccines.



2. Proposed solution to current situation

2.1 Vaccination overcomes access to limitations of diagnostics globally

Chronic infection with HCV is often asymptomatic, thus screening is necessary to identify most patients with infection. The diagnosis of HCV infection relies on detection of antibodies to HCV as well as viral RNA [25]. To notice, HCV treatment can be remarkably effective when HCV is diagnosed in the early stages of the infection and linkages to care are provided. Obstacles vary from one country to another due to economic conditions and the spread of the disease in particular groups. The most important of these is the capability of access to diagnostics especially in low- and middle-income countries. For instance, implementing planned screening programs that target high-risk patients may support the early achievement of the HCV elimination goal. There is a need for low-cost HCV diagnostics to be optimized in low- and middle-income countries especially where fast increasing numbers of exposed persons in a population are present. Facilitating access to screening can generally be achieved by improving serological tests in terms of rapidity and simplicity of performance such as using easy-to-access samples like fingerstick capillary whole blood or oral fluid. Several strategies have been evaluated in last years to simplify and facilitate access to screening and diagnosis as reported in S.*Fourati et al.,* 2017, [26] where the summary of the main characteristics of virological tools used for simplified diagnosis strategies is presented. For instance, some countries (e.g., Georgia, Egypt) have already engaged partners to develop an efficacious prevention and control plan, leading to an improved access to diagnostics and treatment. Significant inputs from public health agencies are necessary to plan screening programs and educate people unaware of HCV.

Even if affluent nations in Europe and North America have low prevalence rates, HCV infections are often undetected, with 80% of infected patients unaware of their infection status [27], as the early stages of HCV infection are in most cases asymptomatic. Therefore, unaware people do not seek medical attention. Indeed, many patients infected with HCV in Germany have not initiated treatment, including a majority of those who are aware of their positive diagnosis [28].

In the USA, the exact number of individuals who are currently infected with hepatitis C virus (HCV), tested by the presence of HCV RNA, is unknown, but it is estimated to be over 2 million people and as many as 3.5 million people. Even in this case, most individuals with HCV infection (approximately 75%) are not aware of their infection. As estimated, 70% to 85% of acute HCV infections become chronic.

Chronic HCV infection is considered the primary reason for liver cirrhosis and hepatocellular carcinoma and the leading cause of liver transplantation. In the USA, HCV causes nearly 40% of all chronic liver disease and is among the most common indications for liver transplantation [3,4,29].

To help in the diagnostics, especially when the prevalence of chronic hepatitis C is low in most countries where making mass screening is neither cost effective nor practical, artificial intelligence (AI) has been implemented. In M. Reiser *et al.*, 2021 [30], AI for social medical data was used to identify predictors of undiagnosed hepatitis C virus infections. To advance diagnostics and reduce medical costs, an Artificial Neural Network (ANN) using a self-organizing map [31] was implemented to analyze socio-medical data of 1.8 million insured for predictors of undiagnosed HCV infections. Further validation studies of AI diagnostics models should be considered.

WHO recommends a shift in testing and treatment delivery for HCV patients. General practitioners and nurses at the primary health care should manage the HCV patients rather than the specialists. The point of care (POC) HCV ribonucleic acid (RNA) assay is simplified, and an efficient testing approach is initiated for a quick result to make an immediate clinical management decision. This approach is applicable to the underserved and marginalized populations with limited access to health care.

In W. Liu *et al*., 2021 [32], AI was used for the diagnosis and screening of hepatitis, evaluating the incidence of the disease, classifying the different stages, forecasting the progression, and predicting response to antiviral drugs in CHC patients with a high level of diagnostic performance.

These approaches based on the use of neural networks may allow for a more efficient risk adapted HCV screening. However, further validations of the prediction model are required.
Considering the different cases reported, we can conclude that:
1) A more practical approach that breaks down the overall goal into smaller goals for patient sub-populations, known as micro-elimination, has been discussed. Micro-elimination works to achieve the WHO goals in specific sub-populations, settings, generational cohorts, or geographic areas [28].
2) To successfully initiate a national screening program, an accompanying program to increase awareness is required.
3) AI based approaches need further validations in terms of robustness.
4) A vaccine for HCV, as a prevention measure, is needed for the disease eradication since the HCV has been reported undiagnosed in many cases leading to challenges in surveillance and mass-screening programs.

2.2 Case studies on delivery burden of Hepatitis C treatment

In low-income countries, infectious diseases still account for a large proportion of deaths, highlighting health inequities caused by economic differences. Vaccination has been proven to be able to cut health care costs and reduce these inequities [33]. “The WHO estimates that two to three million deaths are prevented each year through immunization against diphtheria, tetanus, whooping cough, and measles. Nevertheless, the WHO also estimates that vaccine preventable deaths are still responsible for 1.5 million deaths each year” [34].

Here we present the different faces of the cost of the illness:

1) Less than 25 % of people who are infected with HCV can clear without treatment. However, when treatment is needed, antiviral drugs for hepatitis C are highly effective. Hepatitis C drugs had initial high costs due to the huge demand, expense of bringing them to marketplace, and industry’s need to recoup R &D investments, further enhanced by the lack of a national health care system to negotiate medication costs on behalf of consumers [35] outside of the US Military or Medicare system. Larger purchasing pools have helped health plans, together with the availability of generic DAAs, to reduce the cost of therapies to consumers and through greater medication access provided excellent patient outcomes.

2) The health care market in the United States is consumer-driven due to the free-market economy.This consumer-driven health care market is sensitive to patient demand in determining which drugs are purchased.

   Consumers frequently make decisions about which drugs are preferred based on access to information about drug innovation. Consequently, the consumers in the United States choose newer drugs with greater frequency than in other countries. The increase in demand for Sovaldi™ and then Harvoni™ use, mirrors this trend.

3) J. A Kwon *et al.*, 2021[36] shows that treating HCV-infected prisoners is highly cost-effective.Additionally, it is worthy of mention that, in "Follow California's Lead: Treat Inmates With Hepatitis C", *Health Affairs Blog*, 2018, 20186 [37], the report indicated that California passed a budget allocating $105.8 million to treat 22,000 inmates with HCV incarcerated in its correction system.

4) It is well documented that HCV, if left untreated, can lead to serious and costly health problems, including cirrhosis, liver cancer, liver failure, liver transplant and even death.

5) In M.J. Barber *et al.*, 2020 [38], the authors summarize originator prices for sofosbuvir, daclatasvir, sofosbuvir/ledipasvir, sofosbuvir/velpatasvir, and glecaprevir/pibrenatsvir in 50 countries where publicly accessible price databases were obtained. For instance, the median originator price of sofosbuvir was US $40,502 per 12-week course, ranging from US $10,730 in Argentina to US $91,461 in Italy. The median price of daclatasvir across all countries was US $26,928 per 12-week course, ranging from US $3144 in Russia to US $100,415 in Italy. The most expensive drug relative to GDP per capita resulted in sofosbuvir/velpatasvir in Lebanon, which costs 5.9 times the GDP per capita. Remarkably, prices for DAAs were shown to not be adjusted based on country income level or potential patient population. Thanks to the WHO, low- and middle-income countries can aim to achieve a price as low as US $ 60 per patient for a 12-week course of treatment with WHO-prequalified generic sofosbuvir and daclatasvir. Prices offered by suppliers of WHO prequalified HCV rapid diagnostic tests ranged between US $ 1 and US $ 8 per test. However, many countries still have no access to these low prices [39].

   The existing WHO regional models do not capture the significant heterogeneity between countries within the same region, including differences in epidemic characteristics, prevention, population groups,costs, and existing health systems and in particular the differences between high-income and low-income countries.

   In all the epidemiological HCV studies, countries with missing data are excluded from regional averages, biasing projections towards the epidemiological situations of countries that have better surveillance and reporting systems. On the other hand, a large part of the literature has focused on costs and economic benefits of HCV elimination. For instance, the economic modelling presented in N. Scott *et al*., 2020 [40] provides evidence that a finite period of investment in HCV could generate a net economic benefit of $22·7 billion globally by 2030 and lead to considerable reductions in transmission and mortality, with lower ongoing costs. In **Fig.2**, we present the net economic benefit which was estimated.

Vaccination is recognized as a substantial preventive measure that improves health and allows individuals to contribute to economic growth. However, there are several gains provided by vaccinationand are ignored by traditional economic analyses. Interestingly, in M. Toumi *et al.*, 2015 [41], the impact of vaccines is also discussed in economic terms with the key sentence “why prevention is wealth”.

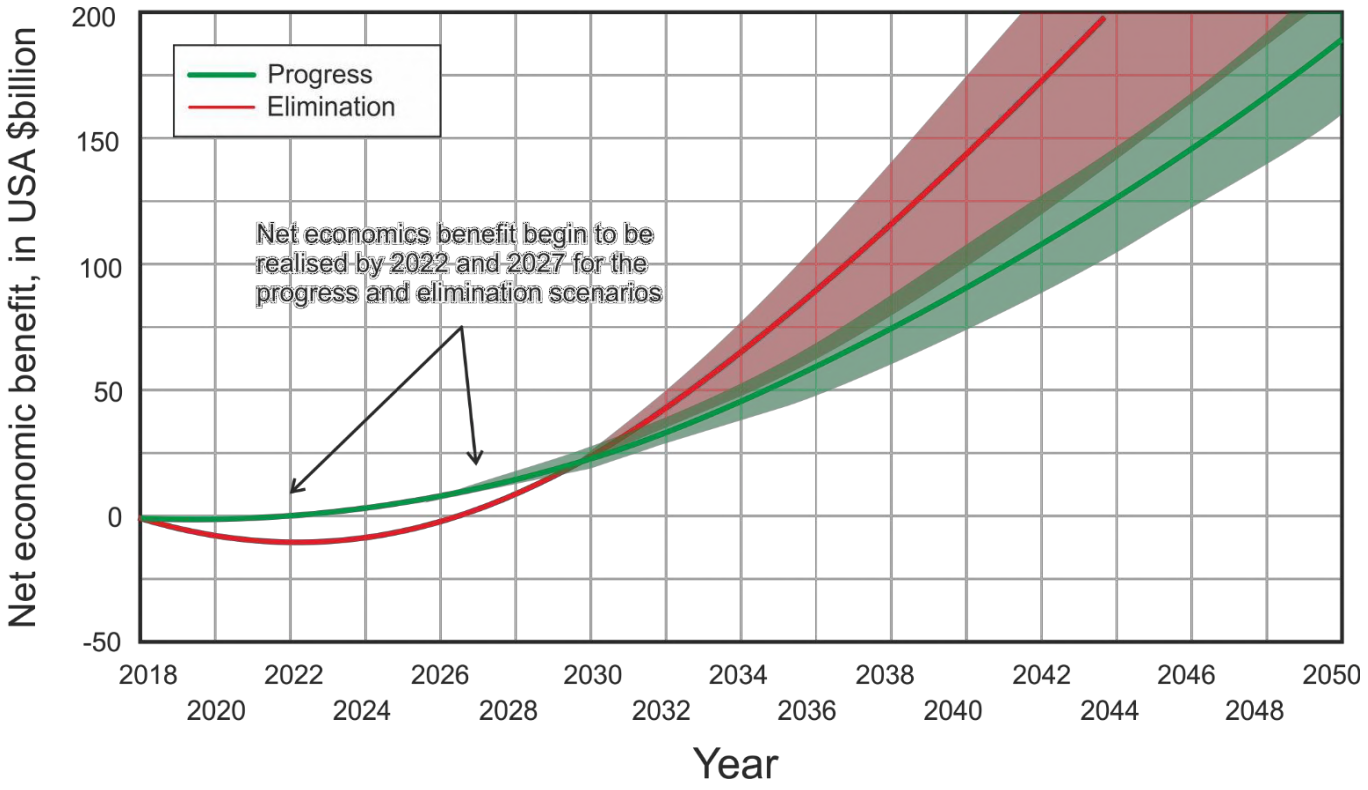


Fig.2 Net economic benefits estimated by 2030. Adapted from *N. Scott et al.*, 2020 [33]. Data were aggregated over the six WHO world regions.
The net economic benefits of the progress and elimination scenarios were calculated as (cumulative testing, treatment, health care, and lost productivity costs in the status quo) – (cumulative testing, treatment, health care, and lost productivity costs in the investment scenario [progress or elimination]). All costs included discounting at 3% per annum. An upfront investment was also considered, and it results in negative benefits (below $0).

Among the different strategies proposed for implementing cost-effective measures/programs for Eradication of HCV and to decrease the burden in the health care systems [35], vaccination is one of the most cost-effective interventions that contributes to healthcare system efficiency.

Here, the revised bibliography highlights that immunization allows reductions in healthcare costs, decreasing lost labor force productivity, thus contributing to social and economic development. R. Deogaonkar *et al.*, 2012[42] shows that several broader categories of economic benefits associated withvaccination in many low- and middle-income countries (LMICs) are captured in primary studies and quantified in economic evaluations. The research identified 26 studies which assessed at least one broader benefit of an immunization program. However, further work on techniques to value such categories and combine them in terms of economic evaluations is still needed.

It is also important to mention the partial eradication of smallpox, the elimination of polio from most

continents, and the control of other diseases, including diphtheria, tetanus, pertussis, rubella, and hepatitis B [43]. It is also worth mentioning the estimated total annual paralytic polio cases prevented since 1950,using the no vaccine scenario in comparison with either the global RC (solid black curve) or the reported cases (dotted purple curve) as shown in **Fig.3**. This single case provides a remarkable demonstration of the substantial impact of vaccines on human health.

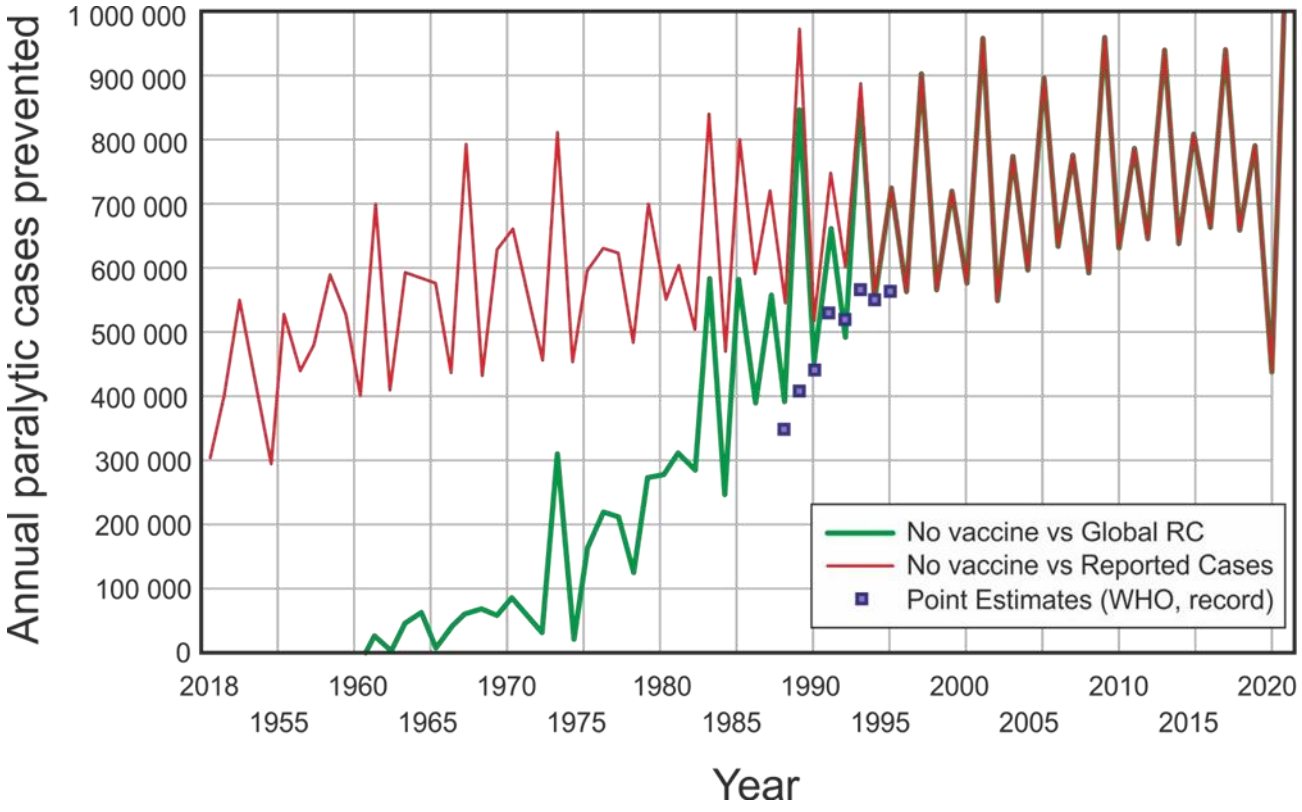


Fig.3 Visualization of the role of vaccines in prevention of paralysis: remarkable demonstration of the substantial impact of vaccines on human health. Figure re-adapted from K. Badizadegan *et al*., 2022 [44]. Black diamonds show WHO published point estimates of the paralytic polio cases prevented for the years 1988 to 1995. Abbreviations: RC, reference case; WHO, World Health Organization

3.HCV 2023 elimination plan in USA

The success of Egypt's 100 Million Healthy Lives campaign provided an excellent model [44] for creating a hepatitis elimination plan throughout the country and illustrated the president's commitment to eliminating hepatitis. In March 2023, the Biden-Harris administration proposed to Congress in its own National Hepatitis Elimination Program [46]. Unlike Egypt, the United States does not possess a national health service and as a result, standardized protocol for surveilling, identifying, and treating hepatitis C is not available. We have previously discussed that, despite the CDC's universal testing guidelines, 40% of the United States' population is unaware of their asymptomatic infection. Furthermore, multilevel barriers at the patient, provider, and system levels are known to contribute to difficulties with access to HCV testing and treatment. Barriers to care has been the object of different research studies especially for addressing disparities in HCV care and treatment [47]. We can here cite two example research cases: 1) population studies that address demographic-based barriers (e.g., age, race, and income) and insurance-based barriers in federally qualified health centers as the one recently discussed in D. Lam *et al.*, 2022 [48], 2) different studies on identifying and removing barriers to treatment promoted by the U.S. Department of Veterans Affairs [49].

The USA Elimination Program plan has aimed to "increase access to curative medications and expand implementation of complementary efforts such as screening, testing, and provider capacity." The plan tackles the principal elimination barriers: the will, the public awareness, the infrastructural organization, and the cost [50].

The recent 2023-report from the CDC, published in the CDC's Morbidity and Mortality Weekly Report indicates that despite the availability of DAAs, only about one-third of people with hepatitis C viral infection (HCV) are achieving viral clearance. The following quantitative data have been presented [24,51]:

- A total of 1.7 million people were identified as having HCV between 2013 and 2021.
- The deaths of more than 14,800 people in 2020 was caused by the infection.

- By December 31, 2022, viral testing was received by 88% of patients.
- Of those tested, 69% were reported to have initial infection.
- Among those with initial infection, just 34% were classified as cured or cleared, either due to treatment or spontaneous clearance.
- Of those achieving viral clearance, 7% were reported as having persistent infection or reinfection.

The report study underlines that much work is still needed to increase access to DAAs, improve diagnostics and prevention efforts. Furthermore, the CDC press briefing transcript [52] commented on a proposed model to deal with the cost issues that some people called the “Netflix model”. This was successfully piloted in Louisiana and proposed negotiation of medications with the companies that have oral therapy approved by FDA. As stated by Dinah V. Parums, MD PhD in *Medical Science Monitor* [53], there is a current hope that the USA national elimination program will promote public awareness and advance research development of effective vaccine platforms to wipe out HCV.

4.Long-term goals

4.1 The need for an HCV vaccine and broad-spectrum research on virus evolution.

We have previously discussed how vaccines can be pivotal in disease eradication and maintenance. Other considerations can be made concerning the need for vaccines. SARS-CoV-2 (as well as other viruses) showed the great capability of mutation, adapting to their surroundings and more effectively moving from host to host (the so-called spillover events [54]). RNA viruses especially evolve with remarkable rapidity, with rates of mutation and substitution that are many logs higher than those of genes from cellular organisms. Such rapid changing rates mean that RNA virus evolution is highly dominated by the process of mutation [55]. Hence, vaccine development can help for giving insights in understanding and monitoring the ability of a virus to generate successful escape mutations. HCV is considered a rapidly mutating RNA virus, with a mutation rate ranging from 3.5×10^{-5} to 1.2×10^{-4} base substitutions/site/year which is mainly driven by the error prone NS5B RNA-dependent RNA-polymerase [2]. These spontaneous rapid mutations result in a high genetic diversity, resulting in so-called “quasispecies.” Despite the large number of HCV genotypes and subtypes, circulating recombinant forms (CRFs) have been described, yet are seldom seen in patients

[56]. The huge genetic variability of HCV brings discrete challenges to host immune control, to the management of HCV- infected patients, and to the development of pan-genotypic treatments. Previously, in 2017, A. Ansari [57] and colleagues at the University of Oxford and other institutions showed the results of a genome-to-genome analysis which revealed that the HCV genome evolves in response to the genome of the infected individual. Furthermore, F. Nakamura *et al.*, 2022 [58] discussed the emergence of HCV with Resistance Associated Substitution (RAS) as a major problem in direct DAAs treatment. The RASs in the HCV genome are thought to be produced because of spontaneous mutations of the HCV RNA, and some HCV with RASs can result in response to the selective pressure of DAAs. However, the mechanism underlying the spontaneous mutations of HCV is not yet fully understood [59].

More research using Whole Genome Sequencing (WGS) to investigate the relationship between viral diversity and duration of HCV infection is needed to compare the longitudinal evolution of each HCV gene region. Additionally, the role of factors such as recent injection drug use, which displays higher levels of intra-host HCV diversity due to re-infection, mixed infection, and higher variability among founder variants, has not been adequately addressed. Interestingly, results from recent studies revealed that genetic diversity and intra-host viral evolution can be different for recent injection drug users [60].

A minority of patients clear the virus spontaneously during acute infection. On the other hand, most patients are unable to clear the virus and develop viral persistence with an ongoing innate and adaptive immune response. This has led some research to focus on understanding factors that contribute to viral persistence and spontaneous clearance [61,62]. HCV has a strict species tropism for humans, and it has developed several strategies for evading the humoral immune system. The complexity of defining the role of neutralizing antibodies (Nabs) in protection from HCV is increased by differential features of the virus particle linked to changes in the E1–E2 viral envelope proteins or associated host factors, which can substantially alter sensitivity to the Nabs.

Interestingly, using low-temperature electron microscopy, scientists at Scripps Research and the University of Amsterdam report have mapped critical proteins that cover the surface of HCV and enable it to enter host cells [63]. The E1E2 envelope complex of HCV has been extremely challenging to image at high resolution. In this study, the researchers were able to find that a combination of three broadly neutralizing anti-HCV antibodies can stabilize the E1E2 complex in a natural conformation. Unique features of E1E2 in complex with various neutralizing antibodies and small molecule inhibitors were studied by Electron Microscopic Analysis (EM) for understanding its antigenicity and induction of immune response [64]. Having structural details of E1E2 may help researchers in designing vaccines that powerfully elicits antibodies to block HCV infection.

We can conclude that, from a broad-spectrum future perspective, a vaccine for HCV can be a resource for dealing with pathogens which show extraordinary difficulty in studying their envelope protein complex and exhibit high rates of escape mutations.

5.Conclusions

We have explored how vaccination can contribute to disease eradication as a cost-effective approach.The recent achievements in structural biology may help in the design of a vaccine.

Here are the key points of our analysis:

- Undiagnosed HCV cases and attempts for fast screening are challenging, especially if we aim for mass screening. More quantitative and qualitative data are needed for reporting HCV viremic prevalence from 2022 in WHO regions.
- Cost savings of HCV treatments are discussed. Evidence shows that the cost of treating the incarcerated is a major concern but generates a significant return on investment over the long term and reduction of general population transmission once former prisoners are released back into communities.
- The emergence of hepatitis C virus (HCV) with resistance-associated substitution (RAS), produced by mutations in the HCV genome, is a phenomenon of studies.
- Vaccines are important clinical components for eradicating HCV, and these will go hand in hand with existing and future treatments, testing and implementing preventive interventions that change lifestyles, behaviors, and wellness.
- The hard work that has allowed the rapid and effective development of vaccines against SARS- CoV-2 should be a good example of what can be achieved if the efforts are focused on tackling the HCV health burden.

In conclusion, in addition to the current treatment research, allocating funds for the purpose of producing an HCV vaccine boosts the overall research area for hepatitis C development that has been left behind in terms of the arsenal of innovations that can be effectively translated into a public health benefit.

6. References

1) Schnell G, Krishnan P, Tripathi R, *et al.* 2018. Hepatitis C virus genetic diversityby geographic region within genotype 1-6 subtypes among patients treated with glecaprevir and pibrentasvir. *PLoS ONE* 13(10): e0205186. https://doi.org/10.1371/journal.pone.0205186

2) Echeverría N, Comas V, Aldunate F, Perbolianachis P, Moreno P, Cristina J. 2021. In the era of rapid mRNA-basedvaccines: Why is there no effective hepatitis C virus vaccine yet? *World J Hepatol* 13(10): 1234-1268. PMID: 34786164 DOI: 10.4254/wjh. v13.i10.1234

3) Cholankeril G, Ahmed A. 2018. Alcoholic Liver Disease Replaces Hepatitis C Virus Infection as the Leading Indication for Liver Transplantation in the United States. *Clin Gastroenterol Hepatol* 16: 1356-1358. PMID: 29199144 DOI: 10.1016/j.cgh.2017.11.045

4) Piñero F, *et al.* 2018. A changing etiologic scenario in liver transplantation for hepatocellular carcinoma in a multicenter cohort study from Latin America. *Clin Res Hepatol Gastroenterol* 42: 443-452. PMID: 29773419. DOI: 10.1016/j.clinre.2018.03.014

5) Wedemeyer H, Khera T, Strunz B, Björkström NK. 2020. Reversal of Immunity After Clearance of Chronic HCVInfection—All Reset? *Front. Immunol.* https://doi.org/10.3389/fimmu.2020.571166

6) Hamdane N, *et al.* 2019. HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist AfterSustained Virologic Response. *Gastroenterology* 156(8):2313-2329.e7. doi: 10.1053/j.gastro.2019.02.038. PMID: 30836093; PMCID: PMC8756817

7) Polaris Observatory HCV Collaborators. 2016. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2(3):161-176. doi: 10.1016/S2468-1253(16)30181-9. PMID: 28404132

8) Retrieved [2022, November5], from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/hepatitis-c

9) Retrieved [2022,November 5], from https://www.who.int/news/item/24-06-2022-WHO-publishes-updated-guidance-on-hepatitis-C-infection

10) Retrieved [2022, November 5], from https://www.cdc.gov/hepatitis/policy/npr/2022/index.htm

11) Retrieved [2022, November 5], from https://www.hhs.gov/sites/default/files/Viral-Hepatitis-National-Strategic-Plan-2021-2025.pdf

12) Heiza M, Elmola K, Salama B. 2021. Unsafe Practices Associated with HCV Infection Among Adults: A Case Control Study. *Int J Prev Med*.12:60. doi: 10.4103/ijpvm.IJPVM_94_20. PMID: 34447502; PMCID: PMC8356959

13) Papaluca T, Thompson A. 2018. HCV elimination: breaking down the barriers to prison-based care. *HepatomaRes* 4:64. http://dx.doi.org/10.20517/2394-5079.2018.53

14) Di Marco L , La Mantia C , Di Marco V. 2022. Hepatitis C: Standard of Treatment and What to Do for Global Elimination. *Viruses* 14, 505. https://doi.org/10.3390/v14030505

15) Manne V, Ryan J, Wong J, Vengayil G, Basit SA, Gish RG. 2021. Hepatitis C Vaccination: Where We Are and Where We Need to Be Pathogens. 14;10(12):1619. doi: 10.3390/pathogens10121619. PMID: 34959574 PMCID: PMC8705661

16) "The hunt for a vaccine for hepatitis C virus continues" 2021. *The Lancet Gastroenterology & Hepatology*6(4):253. doi: 10.1016/S2468-1253(21)00073-X. PMID: 33714362; PMCID: PMC7952124

17) Retrieved [2023, July 16] from https://www.medpagetoday.com/opinion/second-opinions/105464

18) Wei Ji, Min Zhao, Jikun Zhou, Huanwei Zheng, Naizhe Li, Bihua Han, Wenjiao Yin, Shengli Bi, George F. Gao, Yong Zhang, William JL. 2022. Long-Lasting Virus-Specific T Cell Response with Divergent Features in Self-Resolved and Chronic Hepatitis C Virus Patients 35 Years Postinfection. *Immunohorizons* 6 (3): 191–201. https://doi.org/10.4049/immunohorizons.2200008

19) Romano' L, Zanetti AR. 2021. Hepatitis B Vaccination: A Historical Overview with a Focus on the Italian Achievements. *Viruses* 14(7):1515. https://doi.org/10.3390/v14071515

20) Robert T. 2021. T cell immunity to hepatitis C virus: Lessons for a prophylactic vaccine. *Journal of Hepatology* 74: 220-229 ISSN 0168-8278. https://doi.org/10.1016/j.jhep.2020.09.022

21) Cai C, Samir J, Pirozyan MR, *et al.* 2022. "Identification of human progenitors of exhausted CD8+ T cells associated with elevated IFN-γ response in early phase of viral infection." *Nat Commun* 13, 7543. https://doi.org/10.1038/s41467-022-35281-7

22) Anita YM, *et. al.* 2022. "Characteristics of hepatitis C virus resistance in an international cohort after a decade of direct-acting antivirals". *JHEP Reports* 4, 5. 100462

23) Retrieved [2023, July 14] from https://www.cdc.gov/mmwr/volumes/72/wr/mm7226a3.htm

24) Offersgaard A, *et al.* 2023. "Toward a vaccine against hepatitis C virus". *Science* 380,37-38. DOI:10.1126/science.adf2226

25) Jiang X, Chang L, Yan Y, *et al.* 2021. A study based on four immunoassays: Hepatitis C virus antibody againstdifferent antigens may have unequal contributions to detection. *Virol J* 18, 137. https://doi.org/10.1186/s12985-021-01608-x

26) Fourati S, Feld J J, Chevaliez S, Luhmann N. 2017. Approaches for simplified HCV diagnostic algorithms. *J Int AIDS Soc*.21: 17– 25, e25058, https://doi.org/10.1002/jia2.25058

27) Li HC, Lo SY. 2015. Hepatitis C virus: Virology, diagnosis, and treatment. *World J Hepatol.* 7(10):1377-89

28) Sarrazin, C, Boesecke C, Golsabahi-Broclawski S, Moog G, Negro F, Silaidos C, Patel P, *et al.* 2021. Hepatitis C virus: Current steps toward elimination in Germany and barriers to reaching the 2030 goal. *Health science reports* 4(2): e290. https://doi.org/10.1002/hsr2.290

29) Retrieved [2022, November 5] from https://www.hhs.gov/hepatitis/learn-about-viral-hepatitis/data-and-trends/index.html

30) Reiser M, Wiebner B, Hirsch J, *et al.* 2019. Neural-network analysis of socio-medical data to identify predictors of undiagnosed hepatitis C virus infections in Germany (DETECT). *J Transl Med* 17, 94. https://doi.org/10.1186/s12967-019-1832-4

31) Kohonen T. 1982. "Self-Organized Formation of Topologically Correct Feature Maps". *Biological Cybernetics* 43 (1): 59–69. doi:10.1007/bf00337288. S2CID 206775459.

32) Liu W, Liu X, Peng M, Chen GQ, Liu PH, *et al*. 2021. Artificial intelligence for hepatitisevaluation. *World J Gastroenterol* 14;27(34):5715-5726. doi: 10.3748/wjg. v27.i34.5715 PMID: 34629796 PMCID: PMC8473592

33) Largeron N, Lévy P, Wasem J, Bresse X. 2015. Role of vaccination in the sustainability of healthcare systems. *JMark Access Health Policy* 12;3. doi: 10.3402/jmahp. v3.27043. PMID: 27123188; PMCID: PMC480270

34) Retrieved [2023, August 11] from https://ourworldindata.org/vaccine-preventable-diseases

35) Henry B. 2018. Drug pricing & challenges to Hepatitis C treatment access. *J Health BiomedLaw* 14:265-283. PMID: 30258323; PMCID: PMC6152913

36) Kwon JA, Chambers GM, Luciani F, Zhang L, Kinathil S, Kim D, Thein HH, Botha W, Thompson S, LloydA, Yap L, Gray RT, Butler T. 2021. Hepatitis C treatment strategies in prisons: A cost-effectiveness analysis. *PLoS One* 11;16(2): e0245896. doi: 10.1371/journal.pone.0245896. PMID: 33571196; PMCID: PMC7877645

37) Beckman AL, *et al*. July 24, 2018. "Follow California's Lead: Treat Inmates With Hepatitis C". Health Affairs Blog. DOI: 10.1377/hblog20180724.396136 29. Retrieved [2022, November 5] from https://www.healthaffairs.org/content/forefront/follow-california-s-lead-treat-inmates-hepatitis-c

38) Barber MJ, Gotham D, Khwairakpam G, Hill A. 2020. Price of a hepatitis C cure: Cost of production and current prices for direct-acting antivirals in 50 countries. *Journal of Virus Eradication*. 6; 3(2), 100001, ISSN 2055-6640, https://doi.org/10.1016/j.jve.2020.06.001

39) Retrieved [2022, November 5] from https://www.who.int/news/item/27-01-2021-who-highlights-progress-in-accelerating-access-to-hepatitis-c-diagnostics-and-treatment-in-low-and-middle-income-countries

40) Scott N, Kuschel C, Pedrana A, Schroeder S, Howell J, Thompson A, Wilson DP, Hellard M. 2020. A model of the economic benefits of global hepatitis C elimination: an investment case. *Lancet Gastroenterol Hepatol*. 5(10):940-947. doi: 10.1016/S2468-1253(20)30008-X. Epub 2020 Jul 27. PMID: 32730785

41) Mondher T, *et al.*2015. The Economic Value of Vaccination: Why Prevention is Wealth. *J Mark Access Health Policy* 12;3. doi: 10.3402/jmahp. v3.29414. PMID: 27123186; PMCID: PMC4802700

42) Deogaonkar R, Hutubessy R, van der Putten I, Evers S, Jit M. 2012. Systematic review of studies evaluating the broader economic impact of vaccination in low- and middle-income countries. *BMC Public Health* 16;12:878. doi: 10.1186/1471-2458-12-878. PMID: 23072714; PMCID: PMC3532196

43) Retrieved [2023, February 5] from https://www.cdc.gov/vaccinesafety/ensuringsafety/history/index.html

44) Badizadegan K, Kalkowska DA, Thompson KM. 2022. Polio by the Numbers—A GlobalPerspective. *The Journal of Infectious Diseases, 26* (8), 1309–1318. https://doi.org/10.1093/infdis/jiac130

45) Retrieved [2023, July 14] from https://migrationnetwork.un.org/practice/migrant-access-100-million-healthy-lives

46) Fleurence RL, Collins FS. 2023. A National Hepatitis C Elimination Program in the United States: A Historic Opportunity. *JAMA* 329(15):1251–1252. doi:10.1001/jama.2023.3692

47) Retrieved [2023, July 14] from https://www.pbs.org/newshour/health/biden-wants-to-cure-hepatitis-c-by-2030-these-barriers-have-been-standing-in-the-way

48) Lam D, *et al.* 2022. Barriers to Hepatitis C Virus Care and How Federally Qualified Health Centers Can Improve Patient Access to Treatment. *Gastroenterology Research* 15: 6,343-352. ISSN 1918-2805 print, 1918-2813 online, Open Access

49) Retrieved [2023, July 14] from https://www.research.va.gov/topics/hep-c.cfm

50) Retrieved [2023, July 14] from https://www.forbes.com/sites/williamhaseltine/2023/05/18/how-did-egypt-eliminate-hepatitis-c-in-less-than-one-year/?sh=11791fb430e9

51) Retrieved [2023, July 14] from https://www.ajmc.com/view/hcv-cure-and-clearance-rates-remain-low-despite-new-therapies

52) Retrieved [2023, July 14] from https://www.cdc.gov/media/releases/2023/t0629-hepc-briefing.html

53) Parums DV. 2023. Editorial: Effective Direct-Acting Antiviral Treatments Support Global and National Programs to Eliminate Hepatitis C. *Med Sci Monit* 29: e940519 DOI: 10.12659/MSM.940519

54) Ellwanger JH, Chies JAB. 2021. Zoonotic spillover: Understanding basic aspects for better prevention. *Genet MolBiol*. 4;44(1 Suppl 1): e20200355. doi: 10.1590/1678-4685-GMB-2020-0355. PMID: 34096963; PMCID: PMC8182890

55) Holmes EC. 2009. The Evolutionary Genetics of Emerging Viruses. *Annual Review of Ecology, Evolution, and Systematics* 40:1, 353-372

56) Galli A, *et al*.2022. High recombination rate of hepatitis C virus revealed by a green fluorescent protein reconstitution cell system, *Virus Evolution*, 8(1), veab106. https://doi.org/10.1093/ve/veab106

57) Ansari MA, Pedergnana V, *et al*. 2017. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. *Nat Genet*. 49(5):666-673. doi: 10.1038/ng.3835. PMID: 28394351; PMCID: PMC5873514

58) Nakamura F, Takeda H, Ueda Y, *et al.* 2022. Mutational spectrum of hepatitis C virus in patients with chronic hepatitis C determined by single molecule real-time sequencing. *Sci Rep* 12, 7083. https://doi.org/10.1038/s41598-022-11151-6

59) Palladino C, Ezeonwumelu IJ, Marcelino R, *et al*. 2018. Epidemic history of hepatitis C virus genotypes and subtypes in Portugal. *Sci Rep* 8, 12266. https://doi.org/10.1038/s41598-018-30528-0

60) Montoya V, Howe AYM, Dong WY, *et al.* 2021. Intra-host evolutionary dynamics of the hepatitis C virus among people who inject drugs. *Sci Rep* 11, 9986. https://doi.org/10.1038/s41598-021-88132-8

61) Khattab MA, Zakaria Y, Sadek E, *et al.* 2023. Detection of hepatitis C virus (HCV) RNA in the

peripheral blood mononuclear cells of HCV-infected patients following sustained virologic response. *Clin Exp Med* 23, 131–140. https://doi.org/10.1007/s10238-022-00791-7

62) Skinner NE, *et al* .2021. Convergent antibody responses associated with broad neutralization of hepatitis C virus and clearance of infection. *medRxiv*. doi: https://doi.org/10.1101/2021.09.11.21263416

63) Torrents de la Peña A, Sliepen K, Eshun-Wilson L, Newby ML, Allen JD, Zon I, Koekkoek S, Chumbe A, Crispin M, Schinkel J, Lander GC, Sanders RW, Ward AB. 2022. Structure of the hepatitis C virus E1E2 glycoprotein complex. *Science* 21;378(6617):263-269. doi: 10.1126/science. abn9884. PMID: 36264808.62

64) Kanai T, Hu Z, Yang R, Wu W, Wang Z, M. Houghton, *et al*. 2023. Three-Dimensional Reconstruction of the Hepatitis C Virus Envelope Glycoprotein E1E2 Heterodimer by Electron Microscopic Analysis. *Journal of Virology* 97(1), e01788-22

7. Authors Biographies

Benedetta Pelosi, MS is an Italian researcher in molecular biology formed between University of Florence and Sweden. In particular, she attended the Master program "Plant and Forest Biotechnology" at Umeå University from 2014 to 2015. Subsequently she has been in a Molecular Biosciences program at the Wenner-Gren Institute, Stockholm University as a PhD (c) and obtained a licentiate degree in early 2022 with a work on computational biology focused on a filtering pipeline for cataloguing proteins and genes. Additionally, she is fully certified on Comprehensive Training for Promotionof Vaccine Demand to Maintain and Restore Routine Immunization and Promote Covid-19 Vaccination by WHO, CDC, UNICEF and GAVI.

Michael Houghton, Hon. Laureate, PhD, along with Chiron Corp colleagues Qui-Lim Choo and George Kuo together with CDC collaborator Dan Bradley, identified the hepatitis C virus (HCV) in 1989 after an intensive 7 year search for this elusive virus. Over the next few years, Dr. Houghton and colleagues developed a series of HCV blood tests that resulted in the complete prevention of HCV transmission following blood transfusion. Armed now with a knowledge of the viral replication strategy and efforts from the global academic, private, & public sectors, highly potent and safe antivirals were developed over thenext 25 years that can now cure all HCV patients. The missing link is now a vaccine that would effectively eliminate the threat of HCV infection to humans. Houghton and his previous and new colleagues at the University of Albertain Edmonton, Canada are taking HCV vaccine candidates into the clinic over the next 2 years. These and other candidatesfrom other groups are likely to lead to an approved HCV vaccine by 2030 thus ending the global pandemic from HCV infections.

Patricia Perkins, MSPH, AEMT, is an emergency management and infectious disease research program administrator. Her expertise includes program development for clinical research in pediatric asthma, childhood immunization, HIV-TB,HCV, and liver transplant programs. She maintains active membership in AASLD (liver disease), and APHA (public health) as a presenter and research abstract reviewer, and as a chair or co-chair of conference sessions on community primary and infectious disease medical care delivery.

William M. Remak, MPH, MS, MT, a cancer and two-time an organ transplant recipient, global patient advocate for chronic/infectious diseases, medical research, and vaccine literacy. Founder, Chair of the California Hepatitis C Task Force, Preceptor for public health, and related programs affiliated with several prominent universities.

Dr. Zahra M. Seid MD, MPH, DrPH, with a demonstrated history of working in the non-profit humanitarian organizations (WHO/UNICEF) in the clinical and preventive health industry. She is skilled in Epidemiology, Prevention, Healthcare, Global Health, and Medicine. Dr. Seid is a strong healthcare management professional with a medical doctor and public health delivery background. Dr. Seid has an interest in promoting UNICEF's mandate for a disease prevention program in marginalized communities.

Maia Romanowska, IM, is an experienced project manager and public health specialist working in the non-profit organizations (SHL/Epi-Guard/GIANT) in the AMR/immunization sectors. Skilled in public health, leadership, disease prevention and control, implementation research, immunization, social media, and global health. Infodemic Manager accredited by WHO,GAVI and UN. Global Pandemic Action ambassador with interest in immunization and one health promotion