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REVIEW ARTICLE

Genetic Variability, Infection Kinetics, or Resistance-Associated Substitutions: Which One is Big Elephant in the Room to Eliminate Hepatitis C Virus by 2030

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ABSTRACT

Hepatitis C virus infection kinetics extrapolate complex crosstalk between viral proteins and host cell interactions by involving a plethora of genes and cell signaling to escape host immune responses and subsequently exacerbate the infection course. The approvals and the availability of oral, highly efficacious, and pan-genotypic direct-acting antivirals to treat chronic hepatitis C infection have raised the hopes of achieving the World Health Organization's ambitious goal of hepatitis C elimination by 2030. Several pitfalls exist in the current continuum of HCV care and certain gaps need to be filled for the successful implementation of HCV treatment and elimination strategies in highly endemic and vulnerable populations in many parts of the world in particular low to middle-income countries. This review aims to explore to what extent and to what capacity HCV kinetics, treatment-induced resistance associated substitutions (RASs), and crosscutting barriers of HCV genetic variabilities will impede the efforts and progress toward its elimination.

INTRODUCTION

Worldwide 58 million people are living with the hepatitis C virus (HCV) and Asia, Sub-Saharan Africa (SSA), and South and Central America are home to 31, 10, and 3.5 million people infected with HCV respectively[1,2]. The infection and associated comorbidities (e.g., hepatic fibrosis, cirrhosis, and hepatocellular carcinoma) are still major health problems and significantly impact healthcare resources and services in highly prevalent countries[3]. The incidences of new infections are also escalating despite the advancement in HCV screening, diagnostic algorithms, and the availability of highly efficacious, well-tolerable, and relatively safe therapeutic regimens[3]. The global hepatitis C report estimates 1.75 million new infections in 2015 and a half million deaths in 2017 which indicates a global incidence of HCV infection of 23.7 per 100,000 individuals[2,3].

Hepatitis C virus-host cell interactions are always phenomenal to our understanding of the molecular pathogenesis of virus infection and its progression to chronic hepatitis C (CHC) and further insidious extrahepatic clinical manifestations[4]. Certain aspects of hepatitis C viral kinetics have been explored but even more remains to elucidate in terms of viral quasispecies, genome heterogeneity, and its pivotal role in treatment failure[4]. The use of highly efficacious direct-acting antivirals (DAAs) to treat chronic hepatitis C (CHC) infection and associated comorbidities is promising in real-world clinical settings; however, the emergence of treatment-induced RASs against certain HCV genotypes (GTs) is increasing and compromising the cure in patients with associated comorbidities (e.g., HCV patients with decompensated cirrhosis, HCV/HBV or HCV/HIV co-infections, HCV patients with chronic kidney failure, etc.)[3]. Furthermore, the high price tags, availability, and accessibility of these regimens in some parts of the world also represent inequity for treatment to all infected with HCV[4]. HCV cascade of care is also complicated and not in reach to highly vulnerable and out-of-reach HCV-affected populations. The availability of rapid-diagnostic tests (RDTs) and point-of-care (POC) testing for HCV screening and diagnosis for further management is also scarce in low to middle-income countries and even not fully implemented in resource-replete nations[5]. All these factors reflect some cross-cutting barriers to surmount while achieving the WHO HCV elimination goal by 2030 both at micro and macro-elimination levels[5].

This timely review intends to highlight how the HCV infection kinetics play a role in the propagation of acute infection to chronic disease and irreversible hepatic comorbidities (decompensated cirrhosis, hepatocellular carcinoma) as well as how the genetic variabilities of the HCV genome evolve various viral GTs/subtypes and exhibition of viral quasispecies behavior. The review also highlights how the emergence of treatment-induced RASs affects the overall cure rates

of HCV infection worldwide and lastly how HCV genome heterogeneity is obstructing to achievement of the global goal of HCV elimination by 2030.

HCV Infection Kinetics are quite cumbersome and persist long

HCV mainly replicates in the human hepatocytes; however, some studies also depict HCV lymphotropic as an essential phenomenon for infection progression by evading human immune system responses[6]. HCV acute infection serology varies from one person to another but viral genome (single-strand positive sense RNA genome) detection in the plasma/serum of a patient confirms HCV infection[6]. The early clinical manifestations of acute hepatitis C infection represent elevated ALT (alanine transaminase and AST(aspartate transaminase)) levels, fever, and jaundice[6]. Several studies also unveil that HCV RNA may persist for several years even if the acute infection is naturally resolved in an infected person[6]. Usually, the symptoms of acute infection appear one to three months after exposure to HCV and last two weeks to three months. If the acute HCV infection persists without any natural resolution or is not managed clinically, it progresses toward chronic hepatitis C (CHC) in 80% of acutely affected individuals[6]. The clinical manifestations of CHC propagate slowly and are characterized by persistent hepatic inflammation and necrosis[7]. HCV RNA in the sera of CHC-infected patients persists and is continuously detected throughout the infection. HCV-neutralizing antibodies (HCV-nAbs) remain positive for years; however, some studies report a spontaneous decline in HCV-nAbs levels during CHC infection or after treatment[7]. Liver biochemistry abnormalities in particular fluctuations in serum AST and ALT levels are the hallmark of CHC infection and correlate to CHC progression into hepatic inflammation and necrosis. Hepatic inflammation further progresses toward liver fibrosis and cirrhosis in 10-20% of CHC patients within 20-30 years[7]. During this period, HCV RNA replication (also known as viral load/titer) and translation (viral polyprotein processing) increase many times in advanced hepatic manifestations (e.g., hepatic fibrosis and cirrhosis), and infection progression becomes unpredictable. Hepatic cirrhosis (compensated at that time) remains slothful for some CHC patients; however, by in large converts to an irreversible form (decompensated state) in 2-3% of CHC-affected individuals which further leads to hepatic carcinoma (known as hepatocellular carcinoma (HCC)) and ultimately death[6,7].

The existing clinical data and experimental evidence suggest very high replication kinetics (10^{12} virions/day) and an estimated 10^{-3} to 10^{-5} mutations/nucleotide per replication cycle of HCV[3,8]. The very rapid replication rate and error-prone nature of the HCV replication enzyme (RdRp; RNA-dependent RNA polymerase) are considered the pivotal factors for HCV genetic variabilities responsible for the evolution of many HCV genotypes, numerous subtypes, and viral quasispecies dynamics[8]. An acute HCV-infected person may comprise 10^{9}-10^{12} virions at any point of the infection status. However, CHC-infected individuals may experience very high levels of virus load (i.e. 10^{5}- 10^{7} virions/mL) [8]. It means that viral RNA in an HCV-infected individual with mutation rates of 10^{-3} to 10^{-5}/replication cycle may accumulate around 0.3-1.2 nucleotide substitution in a single infected hepatocyte[3].

Genome diversity: A pretty mind-boggling aspect of HCV Molecular Virology

The molecular epidemiology and evolutionary dynamic studies of the HCV genome reveal that selective survival pressure, recombination, and genetic drift/shift generate two classes of HCV genome diversities[9,10]. The one is often termed an "HCV quasispecies" demonstrating the intra-host variability of viral genome within an infected individual, whereas the second one deciphers the global prevalence of HCV GTs and multiple subtypes[11,12]. From the evolutionary point of view, HCV quasispecies refers to the self-organization and adaptability of primitive HCV replicons as a fundamental step for survival in a certain favorable or disadvantaged environment[10]. HCV quasispecies dynamics are estimated to be over 13%; although largely believed not to be uniformly distributed through the genomes of all known HCV quasispecies[13,14,15]. HCV progeny like other rapidly evolving RNA viruses is diverse and unique relative to its progenitors due to the highly error-prone nature of RdRp[15]. The intra-host variability of the HCV genome significantly impacts virus tissue tropism, viral transmission, virus prevalence, viral pathogenesis, and host immune system evasion strategies[14]. Indeed, intra-host variations demonstrate both viral gene function modalities and evolutionary rates between different genes of the HCV genome. For example; HCV genome encoding envelope glycoproteins (E1 and E2) express higher genome diversities because they remain under the continuous and intense selection pressures of both humoral and adaptive immune responses of the infected individuals[14,15]. In contrast, HCV non-structural proteins (e.g., NS5B) reflect lower gene diversities because the selection pressures are predominantly cellular[13]. Higher intra-host HCV genome diversities have been documented for injection drug users (IDUs) because of the higher risks of reinfection and the chances of mixed co-infections (e.g., HCV/HIV), and among the founder patients of HCV viral variants[11,12]. However, it is interesting to mention that intra-host evolutionary dynamics of HCV behave in a clock-like fashion among vulnerable HCV populations (IDUs, sex workers, and HCV/HIV co-infections)[9,10]. It could be a reason that current oral interferon (IFN) free DAAs do not offer long-term immunity against IDUs instead of providing higher efficacies while achieving sustained virologic

response (SVR) rates[9,10]. Multiple studies reveal the occurrence of reinfection incidences in IDUs even after achieving higher SVR rates with IFN-free DAAs[9,10,12,13]. It reflects that despite high cure rates with DAAs, protective immunity is limited or does not exist at all with these regimens. Hence, an effective protective vaccine would be an asset to prevent HCV transmission and its complete elimination while taking into account these genetic differences in the HCV genome[9,10]. On the other hand, HCV genetic diversity both at inter- and intra- host levels may continue to evolve new viral lineages which may increase the viral infectivity as well as the high genetic barrier of resistance to current DAAs[11,12]. However, the measurements of HCV genome diversities and the resulting metrics are very helpful in choosing the individual treatment levels and regimen choices for vulnerable HCV populations[12,13]. Similarly, measures of HCV genome diversities may be helpful to predict an accurate duration of infection and subsequently epidemiological-derived estimates of viral incidence and in turn to establish guidelines for virological surveillance, care, and treatment policies[14,15]. Unfortunately, little is known about HCV immunopathology which involves a plethora of genes and cell signaling to orchestrate these HCV-host interactions[3,15]. An ample understanding of intricate crosstalk between HCV and host immune responses will be integral to designing and developing optimal prophylactic or protective anti-HCV vaccine strategies[15]. Furthermore, the lessons learned recently while combating the lethal variants of the coronavirus disease-19 (COVID-19) pandemic worldwide demonstrate that the development and deployment of an effective prophylactic or protective HCV vaccine would be an asset to vanquishing hepatitis C by 2030[3].

On the other hand, the exhibition of extensive inter-host HCV genome diversities classifies the virus into eight GTs and more than 100 subtypes worldwide[3,15]. Some HCV subtypes represent genetically conserved epidemic lineages and are worldwide distributed; while some others are more localized but highly diverse endemic lineages[3]. HCV GTs differ 30-35% from each other based on their nucleotide sequences and HCV subtypes (sometimes also called "sub-genotypes) depict 10% differences in their nucleotide sequences from each other[3]. Interestingly, the global distribution of HCV GTs is highly heterogeneous and this high genetic variability provides the basis for the evolutionary origins of HCV subtypes[3]. Similarly, the variable distribution of HCV subtypes in the world may be categorized into epidemic and endemic subtypes[3,14]. HCV epidemic subtypes are genetically conserved lineages (sequences) and are worldwide distributed; while HCV endemic subtypes are more localized in Africa and Asia but represent highly diverse lineages[14]. For example; 10 HCV epidemic lineages are well studied in clinical trials of DAAs, however; some highly prevalent endemic lineages were poorly characterized in terms of clinical perspective and paradoxically not well sampled for investigating the computational genomic data[14,15]. By in large, the clinical trials of the most currently approved anti-HCV DAAs were conducted in resource-rich nations (e.g., China, Europe, USA) where HCV epidemic lineages (e.g., HCV subtypes 1a, 1b, 2a, 3a, 4a) are the highly prevalent, and where DAAs' efficacies trials are well studied and well documented[15]. Ultimately, the DAA regimens found highly effective against these HCV subtypes were named "pan-genotypic DAAs" and guidelines were prepared to simplify the treatment paradigms and propose no HCV genotyping is required at the individual level[14,15]. On the other hand, some HCV endemic lineages more confined to Africa and Asia (e.g., 1l, 4r, 3b) have recently been found to resist DAAs treatment in particular against first-generation NS5A inhibitors (e.g., daclatasvir and ledipasvir)[14,15].

The epidemiological, as well as genome sequence studies of endemic lineages, are highly advocated to design optimal regional HCV treatment strategies and to facilitate WHO 2030 viral hepatitis elimination program[3,14,15]. Surprisingly; the high genetic variability of endemic HCV strains provides evidence of the current HCV evolution in areas where they are identified[14,15]. Similarly, the higher genetic variability increases the genetic barrier of viral resistance against pan-genotypic DAAs[10,13,14]. Although; many endemic HCV subtypes respond well to DAA regimens, some like 1l, 3b, and 4r are reported not to respond significantly[15]. Similarly, some HCV GTs are rare in resource-replete nations but are highly prevalent in LMICs and were not fully accessed in clinical trials of pan-genotypic DAAs investigated against HCV infection treatment[10,14].

HCV Genetic diversity and infection kinetics correlate with each other

A direct correlation has been reported between HCV GTs/subtypes and infection kinetics in certain vulnerable HCV populations including the IDUs, hemophiliacs, men who have sex with men (MSM), and emigrants[15]. A good example is the identification of HCV GT-4 isolates in IDUs from Southern Europe; however, the progenitor strains of this genotype predominantly exist in Egypt and Central Africa[14,15]. Unlike other RNA viruses, the natural phenomenon of recombination either at the intra- or inter-genotypic level is very rarely seen during HCV RNA replication and infection progression[16]. The only significant recombinant strain (i.e. RF_2k/1b intergenotypic recombinant) was reported in St. Petersburg, Russia which spread further to become important from an epidemiological and evolutionary point of view[16]. The earlier data suggested that this unique HCV chimera may had a very low prevalence and was not significant in HCV evolution[16]. However, it was underestimated

because, in Georgia, 20% of all identified HCV subtypes were HCV RF_2k/1b from HCV GT-2 specimens which comprised 72% of the total evaluated samples[17,18]. The intra- and inter-genotypic variabilities of HCV strains impact the clinical outcomes of IFN-free DAAs to a certain extent. However, pre-treatment HCV GTs and subtype identification are not recommended for the administration of pan-genotypic DAA regimens according to the recent HCV treatment guidelines[15,16].

Overall cure rates of currently available pan-genotypic DAA regimens are excellent among all HCV GTs/subtypes; Therefore, pretreatment differentiation of HCV-infected patients based on GTs/subtypes or RF may not be prudent[3,11,12]. Nevertheless, in countries where pan-genotypic DAAs are not easily accessible and affordable, the identification of HCV GTs/subtypes could be more fruitful in deciding the most effective treatment choices from available first and second-generation DAAs rather than pan-genotypic DAAs[11,12]. The clinical studies provide the cure rates data of HCV-infected patients with RF_2k/1b within the limited spectrum of DAAs[17]. Interestingly, the available data indicate that the achieved SVR rates for RF_2k/1b infected individuals were more similar to HCV GT-1 patients than GT-2[19]. Initially, in Georgia when sofosbuvir was available as a sole treatment for HCV in 2015, RF_2k/1b infected patients (undifferentiated from HCV GT-2 at pretreatment) achieved less SVR rates (i.e., 79%) with standard treatment of SOF plus RBV (recommended for HCV GT-2 infected patients) used in combination for 12, 16, or 20 weeks[18]. However, when treatment was administered with standard HCV GT-1 regimens (i.e., SOF+RBV+PEG-IFN (pegylated interferon)) in RF_2k/1b patients differentiated from HCV GT-2, overall SVR rates were increased up to 92%. Furthermore, upon genotyping and sequence analyses, it was found that 17 out of 20 treatment failure patients were RF_2k/1b as those were initially thought to be HCV GT-2 infected[18]. After the introduction of the sofosbuvir (SOF)/ledipasvir (LDV) combination and further pan-genotypic DAAs (e.g., sofosbuvir/velpatasvir) in Georgia, the clinical data showed that both treatments were extremely effective against RF_2k/1b infected patients[18]. It indicates that the identification of "HCV RF" though less prevalent worldwide could be fruitful in choosing the cost-effective treatment in areas where pan-genotypic DAAs are not easily accessible and affordable[17,18,19]. In addition, the surveillance of "HCV RF" in highly prevalent areas may also help to design regional effective treatment strategies, improve treatment responses, and optimize treatment outcomes in the context of worldwide HCV elimination by 2030[3,19].

Treatment-Emergent RASs: A potential caveat to treat all infected with HCV

Nucleotide substitutions or polymorphisms occur very randomly during HCV genome replication and most of them impair the virus genome fitness (i.e., the capability of a virus to how well it replicates)[3,20]. Virus variant clones with RASs possess a fitness advantage in the presence of a DAA regimen; however, most of them are outcompeted by wild-type or prototype HCV clones in the absence of DAAs[20]. This virus fitness with specific RAS probably determines that either the RAS will persist after successful DAA therapy or will exist at baseline in treatment-naïve HCV-infected individuals[20]. The existence of baseline HCV genome polymorphism, pre-existing HCV RASs, and treatment-emergent RASs with DAAs are the main clinical diagnostic considerations for HCV care providers while choosing, deciding, and initiating IFN-free DAA therapy either in treatment naïve, treatment-experienced, or treatment failure HCV patients[20].

Several studies reveal reduced clinical efficacy of DAAs against HCV epidemic subtypes because of polymorphisms within the NS5A gene and it also seems true for the cases infected with endemic HCV strains[11,20]. It was reported against first-generation NS5A inhibitors (e.g., daclatasvir & ledipasvir); however, SVR rates were also recorded less with second-generation NS5A inhibitors (e.g., Pibrentasvir)[3,20,21]. Similarly, RASs associated with NS3/4A inhibitors have also been well documented in endemic HCV strains (Table 1); however, not been fully elucidated in clinical trials. Polymorphism in the HCV RdRp enzyme gene (NS5B) is relatively less common and only S282T polymorphism is well studied to be correlated with reduced clinical efficacy of sofosbuvir (SOF) in epidemic HCV lineages[22,23,24]. Furthermore, S282T substitution was occasionally detected in HCV subtypes 4r and 6l *in vitro* studies; however, its *in vivo* clinical impact is still elusive[20,22]. It is also noteworthy here that RASs alone may not be responsible for treatment failure in HCV epidemic lineages in the absence of other clinical characteristics of the patients[3,25]. Hepatic cirrhosis and a history of previous treatment failure are also pivotal to correlate with DAA resistance[22]. Interestingly, it remains to clinically determine whether these risk factors also contribute to DAA treatment failure with endemic HCV lineages[25]. It is not possible to discuss here in detail the RAS's impact on different DAAs used in real-world clinical settings to treat HCV patients; however, the table below enlists the common RASs with reduced sensitivity to DAAs according to HCV GTs/subtypes.

Table 1: Treatment-emergent RASs against all oral IFN-free and pan-genotypic DAAs

a. RASs associated with NS3/4A protease inhibitors (PIs) acting on the NS3 protein of HCV		
RASs	**NS3/4A PIs**	**HCV Genotype/subtypes**
V36M/A/G/L[26]	Paritaprevir, Asunaprevir, Simeprevir, Grazoprevir, Vaniprevir, Glecaprevir Voxilaprevir	HCV GT-1 (1a/1b) HCV GT-6
Q41R/K[26]	Simeprevir, Vaniprevir Voxilaprevir	HCV GT-1 (1a/1b) HCV GT-3 HCV GT-4 HCV GT-6
V55A[26]	Paritaprevir, Asunaprevir	HCV GT-1 (1a/1b)
Y56H/F/L[26]	Asunaprevir, Paritaprevir, Grazoprevir, Glecaprevir, Voxilaprevir, Sovaprevir	HCV GT-1 (1a/1b) HCV GT-3 HCV GT-4 HCV GT-6
Q80K/L/R/G/H[27]	Simeprevir, Asunaprevir, Paritaprevir, Grazoprevir, Vaniprevir,Voxilaprevir,	HCV GT-1 (1a/1b) HCV GT-3
Q80H/K/L+D168E/ V/Y[27]	Asunaprevir, Simeprevir	HCV GT-1 (1b)
Q80K/R+R155K[27]	Simeprevir, Paritaprevir	HCV GT-1 (1a/1b)
Q80K/R/L+D168V/ Y/E[27]	Asunaprevir Simeprevir	HCV GT-1 (1a/1b)
Q80K+V170T Q80L/R/K+R155K/W Q80R+A156V S122G/R+R155K[27]	Simeprevir Simeprevir Simeprevir Simeprevir	HCV GT-1 (1a/1b) HCV GT-1 (1a/1b) HCV GT-1 (1b) HCV GT-1 (1a/1b)
R155K/Q/W+D168/A/ N/V/H/E[27]	Simeprevir Grazoprevir Vaniprevir	HCV GT-1 (1a/1b)
R155K+I/V170T/A[27]	Simeprevir Asunaprevir	HCV GT-1 (1a/1b)
A156V+D168V[26]	Simeprevir Glecaprevir	HCV GT-1 (1b)
Q80K/ R+R155K+D168E[27]	Simeprevir	HCV GT-1 (1b)
b. RASs associated with NS5A inhibitors acting on NS5A protein of HCV		
K/Q/S24R/H/G/N/Q/K[28]	Daclatasvir, Ledipasvir, Ombitasvir, Velpatasvir, Pibrentasvir	HCV GT-1 (1a/1b), HCV GT-3, HCV GT-4
M/L/F/28/A/T/V/G/M/C/I/V/ F/S[28]	Daclatasvir, Ledipasvir, Ombitasvir, Elbasvir, Velpatasvir, Pibrentasvir	HCV GT-1 (1a/1b), HCV GT-2, HCV GT-4
P29S/DELETION[29]	Daclatasvir	HCV GT-1 (1b)
F/M28+Y93H[29]	Daclatasvir	HCV GT-1 (1a), HCV GT-2 (2a/b/c), HCV GT-3a
Q30R+H58D[30]	Daclatasvir, Elbasvir	HCV GT-1 (1a)
Q30R+H58D[30]	Daclatasvir	HCV GT-1 (1a)
A/R30K/Q+L31M+Y93H[31]	Daclatasvir, Pibrentasvir	HCV GT-1 (1b), HCV GT-3a
L31V+Q54H+Y93H L31M+P58L+Y93H[32]	Daclatasvir	HCV GT-1 (1b)
L28I/V+P/T58L/S[33]	Ombitasvir	HCV GT-4 (4a/d)
K24Q+T58P+Y93H[33]	Ombitasvir	HCV GT-4 (4d)
M28T+Q30R+L31V+Y93H[30]	Elbasvir	HCV GT-1 (1a)
S24F+M28K[31]	Pibrentasvir	HCV GT-3a

c. RASs associated with NS5B inhibitors acting on the NS5B protein of HCV		
L159F[34,35]	Sofosbuvir	HCV GT-1 (1a/1b) HCV GT-2 (2a/b/c) HCV GT-3 (3a)
S282 R/T/G[34,35]	Sofosbuvir	HCV GT-1 (1a/1b) HCV GT-2 (2a/b/c) HCV GT-3 (3a) HCV GT-4 (4a/d) HCV GT-5 HCV GT-6
C316F/H/N/Y[36]	Sofosbuvir Dasabuvir	HCV GT-1 (1a)
L320F V321A/I[34,35]	Sofosbuvir	HCV GT-1 (1a) HCV GT-1 (1a) HCV GT-3 (3a)
S368T A395G M414I/T/V C445F E446K/Q Y448C/H/R C451S A553T/V G554S/D S556G/N/R G558R D559G Y561H S565F[37]	Dasabuvir	HCV GT-1 (1b) HCV GT-1 (1a) HCV GT-1 (1a/b) HCV GT-1 (1b) HCV GT-1 (1b) HCV GT-1 (1a/b) HCV GT-1 (1b) HCV GT-1 (1a/b) HCV GT-1 (1a) HCV GT-1 (1a/b) HCV GT-1 (1a) HCV GT-1 (1b) HCV GT-1 (1a) HCV GT-1 (1a)
L159F+C316N L159F+L320F L159F+S282T L320F+S282T L159F+S282T+L320F[38]	Sofosbuvir	HCV GT-1 (1a/1b) HCV GT-2 (2a/b/c) HCV GT-3 (3a) HCV GT-1 (1a/1b) HCV GT-3 (3a) HCV GT-1 (1a/1b) HCV GT-1 (1a) HCV GT-1 (1b)

All DAAs exhibit higher clinical efficacy with excellent SVR rates against HCV GT-1-infected individuals[3]; however, some HCV GT-1 subtypes found highly prevalent in West Africa have been reported with lower SVR rates[25]. Two studies reported from the UK showed that subtype 1l (most prevalent in Nigeria and Cameroon) and some other unclassified HCV GT-1 subtypes poorly responded to DAAs[25]. In another study involving a large number of participants native to West Africa and infected with HCV GT-1 subtypes rarely found in the UK, only 75% achieved SVR rates. In contrast, overall SVR rates were excellent in HCV subtypes 1a and 1b infected individuals[25]. The therapeutic outcomes of DAAs against these HCV subtypes from the ongoing studies in West Africa are still elusive. In vitro, evidence of poor DAA responses against some HCV GT-3 subtypes has also emerged in a Chinese study. In this single-arm phase III clinical trial, non-cirrhotic HCV subtype 3b infected patients were treated with LDV/SOF fixed-dose combination (FDC), and 89% achieved SVR rates while for those with cirrhosis, only 50% acquired SVR12 rates at post-treatment. The clinical trials for DAAs outcomes in patients infected with HCV subtype 3g are under investigation[25].

A single-arm prospective study (i.e., SHARED) conducted in Rwanda evaluated the clinical efficacy of LDV/SOF in adults infected with CHC genotype 4 infections[39,40]. HCV GT-4 subtype 4k is found to be highly prevalent in Gabon, the Dominican Republic of Congo (DRC), Uganda, and Rwanda. 93% of subtype 4k infected patients achieved SVR rates with LDV/SOF FDC[39]. The regimen was widely distributed in Sub-Saharan Africa (SSA) in generic formulations to cure

CHC-infected populations[41]. Overall SVR rates were also found excellent against subtypes 4q and 4v (i.e., 90% and 100% respectively). However, in silico, *in vitro*, and *in vivo* estimates of SVR rates in a small number of participants in European clinical trials predicted far lower than expected (i.e. 56%; 27/48) for HCV subtypes 4r infected individuals[41]. However; the patients infected with non-subtype 4r in the clinical trial demonstrated higher SVR rates (i.e., 92%) in those treated with LDV/SOF combination[41]. HCV subtype 4r following 4k is predominantly found in several SSA countries including DRC, Rwanda, South Africa, Ethiopia, and Malawi. It has also been identified in several HCV isolates from Saudi Arabia in recent years. RASs responsible for HCV subtype 4r strains have also been documented against other NS5A inhibitors (e.g., daclatasvir, velpatasvir, and ledipasvir-based DAA regimens) in small numbers of participants in studies reported from the UK and France[39,41]. LDV/SOF FDC for subtype 4k or 4r infected populations, an ongoing phase IV follow-on study (SHARED3) in Rwanda is aimed to evaluate therapeutic outcomes of SOF and velpatasvir (VEL) as the first line and SOF/VEL and voxilaprevir (VOX) as the second line therapy for HCV subtype 4k or 4r infected populations instead of administering LDV/SOF FDC[42]. Other sparsely populated but important from an epidemiological point of view, subtypes of HCV GT-4 like 4b (DRC), 4c, 4g (DRC and Gabon), and 4l were also a part of SHARED **clinical trials. However; the SVR rates data are scarce for other HCV GT-4 subtypes which are not included in SHARED studies like 4e (Gabon), 4f (Cameroon, Algeria), 4h (DRC), 4m (Egypt), 4o (Saudi Arabia), 4p Cameroon), 4s (Uganda) and 4t (Cameroon)[39,41]. Among all these endemic HCV 4 subtype lineages, *in vitro* resistance against LDV has been demonstrated by subtype 4b and warrants further investigations[41]. Subtype 6a of HCV GT-6 responds well to pan-genotypic DAAs both *in vitro* and *in vivo*; however, limited data from the clinical trials highlight higher SVR rates in HCV subtype 6a infected individuals[3,28]. It would be of paramount interest to evaluate the SVR rates of current DAA regimens against other HCV GT-6 subtypes which are predominantly found in Southeast Asia. *In vitro* analysis of a study explores the emergence of triple RAS motif in HCV subtype 6u and 6v strains against first-generation NS5A inhibitors (e.g., DCV and LDV)[28]; however, real-world clinical data administering first and second-generation NS5A inhibitors and newer anti-NS5A agents (e.g., ravidasvir) are eagerly awaited.**

CONCLUSIONS

Eliminating Hepatitis C virus infection as a public health problem by 2030 is feasible, but it will take substantial efforts, hard work, resources, and policy changes. HCV infection dynamics and its correlation to treatment outcomes need further elucidate both at the molecular and cellular level to prevent HCV-associated comorbidities and for the choice of the right treatment options. Now, we have innovative diagnostic tools to screen HCV patients and treatment in the form of pan-genotypic DAAs which can cure CHC infection in more than 95% of patients. Treatment-emergent RASs against DAAs would be a crossing-cutting barrier to get the full benefits of these therapeutic regimens; although pan-genotypic DAA regimens are highly efficacious against all major HCV GTs and epidemic subtypes. Careful treatment considerations, up-to-date guidelines, and recommendations for HCV surveillance, testing, and "treat those who need it" would be of paramount interest in vulnerable and out-of-reach HCV populations. "One size does not necessarily fit all"; however, worldwide HCV elimination is within our grasp".

Conflict of Interest
The author of the study hereby affirms no potential conflict of interest by any means.
Financial Disclosure
The author states that no financial benefit by any means was obtained for the completion of this study.

REFERENCES

1. **Guntipalli P, Pakala R, Kumari Gara S, Ahmed F, Bhatnagar A, Endaya Coronel MK, Razzack AA, Solimando AG, Thompson A, Andrews K, Enebong Nya G, Ahmad S, Ranaldo R, Cozzolongo R, Shahini E. Worldwide prevalence, genotype distribution and management of hepatitis C. Acta Gastroenterol Belg 2021;84:637-656.**
2. Kassa GM, Walker JG, Alamneh TS, Tamiru MT, Bivegete S, Adane A, Amogne W, Dillon JF, Vickerman P, Dagne E, Yesuf EA, Hickman M, French CE, Lim AG; DESTINE NIHR Global Health Research Group. Prevalence, trends, and distribution of hepatitis C virus among the general population in sub-Saharan Africa: A systematic review and meta-analysis. Liver Int 2024;44:3238-3249.
3. Manns MP, Maasoumy B. Breakthroughs in hepatitis C research: from discovery to cure. Nat Rev Gastroenterol Hepatol 2022;19:533-550.
4. Liu CH, Kao JH. Acute hepatitis C virus infection: clinical update and remaining challenges. Clin Mol Hepatol 2023;29:623-642.
5. Shahid I, Alzahrani AR, Al-Ghamdi SS, Alanazi IM, Rehman S, Hassan S. Hepatitis C Diagnosis:

Simplified Solutions, Predictive Barriers, and Future Promises. Diagnostics (Basel) 2021;11:1253.

6. Sallam M, Khalil R. Contemporary Insights into Hepatitis C Virus: A Comprehensive Review. Microorganisms 2024;12:1035.
7. Tsai WC, Chiang HC, Chiu YC, Chien SC, Cheng PN, Chiu HC. Chronic Hepatitis C Virus Infection: An Ongoing Challenge in Screening and Treatment. Life (Basel) 2023;13:1964
8. Tabata K, Neufeldt CJ, Bartenschlager R. Hepatitis C Virus Replication. Cold Spring Harb Perspect Med 2020;10:a037093.
9. Ahovègbé L, Shah R, Kpossou AR, Davis C, Niebel M, Filipe A, Goldstein E, Alassan KS, Keke R, Sehonou J, Kodjoh N, Gbedo SE, Ray S, Wilkie C, Vattipally S, Tong L, Kamba PF, Gbenoudon SJ, Gunson R, Ogwang P, Thomson EC. Hepatitis C virus diversity and treatment outcomes in Benin: a prospective cohort study. Lancet Microbe 2024;5:697-706.
10. Vo-Quang E, Pawlotsky JM. 'Unusual' HCV genotype subtypes: origin, distribution, sensitivity to direct-acting antiviral drugs and behaviour on antiviral treatment and retreatment. Gut 2024;73:1570-1582.
11. Shah R, Ahovegbe L, Niebel M, Shepherd J, Thomson EC. Non-epidemic HCV genotypes in low- and middle-income countries and the risk of resistance to current direct-acting antiviral regimens. J Hepatol 2021;75:462-473.
12. Mbisa JL, Lapp Z, Bibby DF, Phillips LT, Manso CF, Packer S, Simmons R, Harris K, Mohan J, Chinnappan L, Leitner T, Bradshaw D. Identification of two novel subtypes of hepatitis C virus genotype 8 and a potential new genotype successfully treated with direct acting antivirals. J Infect Dis 2024:jiae253.
13. Tang Q, Chen Z, Li H, Zhang L, Peng M, Zeng Y, Liu X, Liu Z, Hu P. Molecular epidemiology of hepatitis C virus genotypes in different geographical regions of Chinese mainland and a phylogenetic analysis. Infect Dis Poverty 2023;12:66.
14. Athamneh RY, Abudalo R, Sallam M, Alqudah A, Alquran H, Amawi KF, Abu-Harirah HA. Sub-genotypes of hepatitis C virus in the Middle East and North Africa: Patterns of distribution and temporal changes. Infect Genet Evol 2023;109:105412.
15. Gregori J, Ibañez-Lligoña M, Colomer-Castell S, Campos C, García-Cehic D, Quer J. Association of Liver Damage and Quasispecies Maturity in Chronic HCV Patients: The Fate of a Quasispecies. Microorganisms 2024;12:2213.
16. Kassela K, Karakasiliotis I, Kokkiou E, Souvalidou F, Mimidis P, Veletza S, Panopoulou M, Koskinas J, Mimidis K, Mavromara P. Intergenotypic 2k/1b hepatitis C virus recombinants in the East Macedonia and Thrace region of Greece. Ann Gastroenterol 2019;32:88-92.
17. Zakalashvili M, Zarkua J, Gish RG, Zhamutashvili M, Sartania V, Weizenegger M, Bartel J, Raabe M, Gvinjilia L, Metreveli S, Barnova M, Abramishvili N, Rtskhiladze I, Metreveli D. Assessment of treatment options for patients with hepatitis C virus recombinant form 2k/1b. Hepatol Res 2021;51:156-165.
18. Zakalashvili M, Zarkua J, Weizenegger M, Bartel J, Raabe M, Zangurashvili L, Kankia N, Jashiashvili N, Lomidze M, Telia T, Kerashvili V, Zhamutashvili M, Abramishvili N, Hedskog C, Chodavarapu K, Brainard DM, McHutchison JG, Mo H, Svarovskaia E, Gish RG, Rtskhiladze I, Metreveli D. Identification of hepatitis C virus 2k/1b intergenotypic recombinants in Georgia. Liver Int 2018;38:451-457.
19. Zakalashvili M, Zarkua J, Gish RG, Zhamutashvili M, Sartania V, Weizenegger M, Bartel J, Raabe M, Gvinjilia L, Metreveli S, Barnova M, Abramishvili N, Rtskhiladze I, Metreveli D. Assessment of treatment options for patients with hepatitis C virus recombinant form 2k/1b. Hepatol Res 2021;51:156-165.
20. Inzaule S, Easterbrook P, Latona A, Ford NP, Irving W, Matthews PC, Vitoria M, Duncombe C, Giron A, McCluskey S, Lesi O, Tchamgoue S, Halford R, Adda D, Thomson E, Dusheiko G, Jordan MR. Prevalence of Drug Resistance Associated Substitutions in Persons With Chronic Hepatitis C Infection and Virological Failure Following Initial or Re-treatment With Pan-genotypic Direct-Acting Antivirals: A Systematic Review and Meta-analysis. Clin Infect Dis 2024;3:ciae431.
21. Akiyama MJ, Riback L, Reeves JD, Lie YS, Agyemang L, Norton BL, Arnsten JH, Litwin AH. Hepatitis C Resistance-Associated Substitutions Among People Who Inject Drugs Treated With Direct-Acting Antiviral-Containing Regimens. Open Forum Infect Dis 2021;8:ofab474.
22. Itakura J, Kurosaki M, Kakizaki S, Amano K, Nakayama N, Inoue J, Endo T, Marusawa H, Hasebe C, Joko K, Wada S, Akahane T, Koushima Y, Ogawa C, Kanto T, Mizokami M, Izumi N. Features of resistance-associated substitutions after failure of multiple direct-acting antiviral regimens for hepatitis C. JHEP Rep 2020;2:100138.

23. Howe AYM, Rodrigo C, Cunningham EB, Douglas MW, Dietz J, Grebely J, Popping S, Sfalcin JA, Parczewski M, Sarrazin C, de Salazar A, Fuentes A, Sayan M, Quer J, Kjellin M, Kileng H, Mor O, Lennerstrand J, Fourati S, Di Maio VC, Chulanov V, Pawlotsky JM, Harrigan PR, Ceccherini-Silberstein F, Garcia F; SHARED Collaborators. Characteristics of hepatitis C virus resistance in an international cohort after a decade of direct-acting antivirals. JHEP Rep 2022;4(5):100462.
24. Singh AD, Maitra S, Singh N, Tyagi P, Ashraf A, Kumar R, Shalimar. Systematic review with meta-analysis: impact of baseline resistance-associated substitutions on the efficacy of glecaprevir/pibrentasvir among chronic hepatitis C patients. Aliment Pharmacol Ther 2020;51:490-504.
25. Adeboyejo K, King BJ, Tsoleridis T, Tarr AW, McLauchlan J, Irving WL, Ball JK, McClure CP. Hepatitis C subtyping assay failure in UK patients born in sub-Saharan Africa: Implications for global treatment and elimination. J Med Virol 2023;95:e28178.
26. Di Maio VC, Cento V, Lenci I, Aragri M, Rossi P, Barbaliscia S, Melis M, Verucchi G, Magni CF, Teti E, Bertoli A, Antonucci F, Bellocchi MC, Micheli V, Masetti C, Landonio S, Francioso S, Santopaolo F, Pellicelli AM, Calvaruso V, Gianserra L, Siciliano M, Romagnoli D, Cozzolongo R, Grieco A, Vecchiet J, Morisco F, Merli M, Brancaccio G, Di Biagio A, Loggi E, Mastroianni CM, Pace Palitti V, Tarquini P, Puoti M, Taliani G, Sarmati L, Picciotto A, Vullo V, Caporaso N, Paoloni M, Pasquazzi C, Rizzardini G, Parruti G, Craxì A, Babudieri S, Andreoni M, Angelico M, Perno CF, Ceccherini-Silberstein F; HCV Italian Resistance Network Study Group. Multiclass HCV resistance to direct-acting antiviral failure in real-life patients advocates for tailored second-line therapies. Liver Int 2017;37:514-528.
27. McPhee F, Hernandez D, Yu F, Ueland J, Monikowski A, Carifa A, Falk P, Wang C, Fridell R, Eley T, Zhou N, Gardiner D. Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and asunaprevir. Hepatology 2013;58:902-11.
28. Dietz J, Susser S, Vermehren J, Peiffer KH, Grammatikos G, Berger A, Ferenci P, Buti M, Müllhaupt B, Hunyady B, Hinrichsen H, Mauss S, Petersen J, Buggisch P, Felten G, Hüppe D, Knecht G, Lutz T, Schott E, Berg C, Spengler U, von Hahn T, Berg T, Zeuzem S, Sarrazin C; European HCV Resistance Study Group. Patterns of Resistance-Associated Substitutions in Patients With Chronic HCV Infection Following Treatment With Direct-Acting Antivirals. Gastroenterology 2018;154:976-988.e4.
29. Lontok E, Harrington P, Howe A, Kieffer T, Lennerstrand J, Lenz O, McPhee F, Mo H, Parkin N, Pilot-Matias T, Miller V. Hepatitis C virus drug resistance-associated substitutions: State of the art summary. Hepatology 2015;62:1623-32.
30. Hernandez D, Zhou N, Ueland J, Monikowski A, McPhee F. Natural prevalence of NS5A polymorphisms in subjects infected with hepatitis C virus genotype 3 and their effects on the antiviral activity of NS5A inhibitors. J Clin Virol 2013;57:13-8.
31. Fridell RA, Wang C, Sun JH, O'Boyle DR 2nd, Nower P, Valera L, Qiu D, Roberts S, Huang X, Kienzle B, Bifano M, Nettles RE, Gao M. Genotypic and phenotypic analysis of variants resistant to hepatitis C virus nonstructural protein 5A replication complex inhibitor BMS-790052 in humans: in vitro and in vivo correlations. Hepatology 2011;54:1924-35.
32. Poordad F, Felizarta F, Asatryan A, Sulkowski MS, Reindollar RW, Landis CS, Gordon SC, Flamm SL, Fried MW, Bernstein DE, Lin CW, Liu R, Lovell SS, Ng TI, Kort J, Mensa FJ. Glecaprevir and pibrentasvir for 12 weeks for hepatitis C virus genotype 1 infection and prior direct-acting antiviral treatment. Hepatology 2017;66:389-397.
33. Yoshimi S, Imamura M, Murakami E, Hiraga N, Tsuge M, Kawakami Y, Aikata H, Abe H, Hayes CN, Sasaki T, Ochi H, Chayama K. Long term persistence of NS5A inhibitor-resistant hepatitis C virus in patients who failed daclatasvir and asunaprevir therapy. J Med Virol 2015;87:1913-20.
34. Rashed M. Hasen; Nagla Abd Al Monem; Kamal A. Amer; Manal M. El-Gerby. "Biochemical Hepatic Changes in Chronic Hepatitis C Patients Induced by Sofosbuvir and Daclatasvir with or without Ribavirin Regimens", Egyptian Journal of Medical Microbiology, 29, 2, 2020, 137-144. doi: 10.21608/ejmm.2020.250233
35. Xu S, Doehle B, Rajyaguru S, Han B, Barauskas O, Feng J, Perry J, Dvory-Sobol H, Svarovskaia ES, Miller MD, Mo H. In vitro selection of resistance to sofosbuvir in HCV replicons of genotype-1 to -6. Antivir Ther 2017;22:587-597.
36. Tong X, Le Pogam S, Li L, Haines K, Piso K, Baronas V, Yan JM, So SS, Klumpp K, Nájera I. In vivo emergence of a novel mutant L159F/L320F in the NS5B polymerase confers low-level resistance to the HCV polymerase inhibitors mericitabine and sofosbuvir. J Infect Dis 2014;209:668-75.
37. Sulkowski MS, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A,

Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. JAMA 2015;313:1223-31.

38. Sulkowski MS, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A, Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. JAMA 2015;313:1223-31.
39. Grant P, Shumbusho F, Van Nuil JI, Kateera F, Mukherjee J, Kabahizi J, Ntaganda F, Nsanzimana S, Mbituyumuremyi A, Damascene MJ, Muvunyi CM, Mukabatsinda C, Musabeyezu E, Ntirenganya C, Gupta N. Safety and Efficacy of Limited Laboratory Monitoring for Hepatitis C Treatment: A Blinded Clinical Trial in Rwanda. Hepatol Commun 2020;4:569-576.
40. Alaa Aboud; Mostafa S. ABDEL-MOTaleb; Ehab M. Fahmy. "Evaluation of Sofosbuvir plus Ribavirinin Treatment of Cirrhotic Hepatitis C Patients: An Egyptian Study", Egyptian Journal of Medical Microbiology, 28, 1, 2019, 15-22. doi: 10.21608/ejmm.2019.282306
41. Gupta N, Mbituyumuremyi A, Kabahizi J, Ntaganda F, Muvunyi CM, Shumbusho F, Musabeyezu E, Mukabatsinda C, Ntirenganya C, Van Nuil JI, Kateera F, Camus G, Damascene MJ, Nsanzimana S, Mukherjee J, Grant PM. Treatment of chronic hepatitis C virus infection in Rwanda with ledipasvir-sofosbuvir (SHARED): a single-arm trial. Lancet Gastroenterol Hepatol 2019;4:119-126.
42. Gupta N, Manirambona L, Shumbusho F, Kabihizi J, Murangwa A, Serumondo J, Makuza JD, Nsanzimana S, Muvunyi CM, Mukabatsinda C, Musabeyezu E, Camus G, Grant PM, Kateera F. Safety and efficacy of sofosbuvir-velpatasvir-voxilaprevir for re-treatment of chronic hepatitis C virus infection in patients with previous direct-acting antiviral treatment failure in Rwanda (SHARED-3): a single-arm trial. Lancet Gastroenterol Hepatol 2022;7(6):542-551.