Recent Advances in Clinical Trials of HCV Vaccines
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ABSTRACT
Hepatitis C virus infects nearly 3% of the global population, and spreads to 3-4 million new people annually. HCV infection is a leading cause of liver cirrhosis, hepatocellular carcinoma, and end-stage liver diseases and causes liver-related death in more than 300,000 people each year. Unfortunately, there is currently no vaccine for HCV prevention (prophylactic vaccine) or treatment (therapeutic vaccine). Circulating HCV is genetically diverse, and therefore a broadly effective vaccine must target conserved T- and B-cell epitopes of the virus and induce strong cross-reactive CD4+/CD8+ T-cell and neutralizing antibody responses in preventing or clearing HCV infection. So far, a few of vaccine development approaches are successful and some of the HCV vaccine candidates have reached human clinical trials, including those modalities mainly based on recombinant proteins (envelope proteins and core protein subunit), synthetic peptides, DNA (plasmid) and viral vectors (virosome). Encouraging results were obtained for those HCV vaccine formulations consisting of prime-boost regimen involving a live recombinant viral vector vaccine alone or in combination with DNA or subunit vaccine. Among several other vaccine strategies under preclinical development, the most promising one is virus like particle based vaccine that will be moving into human studies soon.

Abbreviations: HCV, Hepatitis C virus; UTR, Untranslated regions; MVA, Modified vaccinia Ankara; VLP, Virus-like particle

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Introduction

Hepatitis C virus (HCV) was initially recognized as the causative agent for parenterally transmitted non-A, non-B viral hepatitis (NANBH) in 1975 [1]. Around 1988, the genome of HCV was cloned as a cDNA without seeing the viral particles themselves [2]. In 2004, an in vitro HCV replication system was established to produce viral particles in the culture medium [3]. Later, more in vitro culturing systems were developed [4]. Together with animal models including chimpanzee and genetically humanized mice, those important tools greatly facilitated study of hepatitis C virus infection and related liver disease [5]. Notably, the chimpanzee model has been proven very useful in preclinical HCV vaccine development [15].

Nowadays HCV is globally distributed and it is estimated that more than 170 million people (about 3% of the world’s population) are infected worldwide with another 3-4 million people newly infected every year. Following acute/primary infection, 20% of people spontaneously eradicate the virus over weeks or months and are often asymptomatic. Most people develop persistent infection, of whom approximately 20-50% develop progressive liver disease leading eventually to liver cirrhosis, liver failure and hepatocellular carcinoma. The current therapy for HCV infection is pegylated interferon and ribavirin (PEG-IFN/ribavirin). This treatment is expensive, prolonged, has extensive side effects and often ineffective. Overall, HCV causes liver-related death in more than 300,000 people annually. Therefore, a vaccine that can efficiently prevent and treat HCV infection is urgently needed. Nevertheless, there is currently no such vaccine available but a number of approaches are in successful development and some of them have reached human clinical trials [6-8].

With a brief introduction on genomic structure and heterogeneity of the virus, this review mainly focus on progress made so far in clinical trials of HCV vaccines.

Virion, genomic structure and diversity of HCV

HCV is a small spherical enveloped virus with a diameter of about 60 nm (Fig.1 I). The virus is non-cytopathic, hepatotropic and a prototype member of the Hepacivirus in the Flaviridae family. The HCV particle consists of a core of genomic RNA surrounded by an icosahedral protective shell of nucleocapsid protein, and further encased in a lipid envelope which originates from the infected host cells. Two viral envelope glycoproteins, E1 and E2, formed heterodimers are embedded in the lipid envelope (Fig. 1 II).

The genome of HCV is a positive-sense single-strand RNA which is roughly 9.6 kb and contains one single open reading frame, and the 5' and 3' ends of the RNA are the untranslated regions (UTR) (Fig. 2).
single open reading frame is translated into a single polyprotein of 3010 amino acids, which is then further cleaved by host endogenous proteases or viral encoded proteases (NS2 and NS3) to produce 10 smaller mature and active proteins (Fig. 2). The N-terminal part encodes the structural elements core (C)—the forming unit of the capsid and the envelope glycoproteins E1 and E2. Both E1 and E2 are highly glycosylated and important in cell entry. Conserved B-cell epitopes inducing /recognized broadly neutralizing antibodies were already identified in E1 and E2. In addition, E1 serves as the fusogenic subunit and that E2 acts as the receptor binding protein. The C-terminal part comprises seven non-structural proteins including NS3 to NS5B responsible for HCV genome amplification. p7 and NS2 are non-structural protein dispensable for replication, but essential for viral assembly. Core and NS3 contain the most conserved and protective epitopes for eliciting cellular responses.

Based on the diversity of the genome, HCV is classified into six or seven major genotypes (with >30% overall sequence difference) and a number of different subtypes within each genotype (with 10-25% sequence difference and more than 50 sub-genotypes) [9]. The geographical distribution of HCV genotypes differs as demonstrated by epidemiologic studies. HCV genotypes 1, 2, and 3 are found worldwide and genotypes 1 and 3 are the most widely distributed. Genotype 4 is predominant in Egypt and genotype 5 is mainly widespread in South Africa, genotype 6 is more prevalent in Southeast Asia [11].

Similar to another well known RNA virus HIV (human immunodeficiency virus), HCV displays high genetic diversity within and between infected individuals and even a single infected host harbors a large

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**Fig 1.** I. Electron micrograph of hepatitis C virus purified from cell culture: black bar=50 nm (Adapted from [http://upload.wikimedia.org/wikipedia/commons/6/6e/HCV_pictures.png](http://upload.wikimedia.org/wikipedia/commons/6/6e/HCV_pictures.png)).

II. Structure of the HCV particle (Adapted from Hepatitis C Virus Database [http://www.hcvdb.org](http://www.hcvdb.org)).
number of quasispecies. The great heterogeneity of HCV is driven by the poor fidelity of RNA polymerase and rapid turnover of the virus as confirmed by single genome sequencing [12]. It is estimated that an individual produces as many as $10^{12}$ virions per day [13], and the median in vivo viral mutation rate is $2.5 \times 10^{-5}$ mutations per nucleotide per genome replication during primary infection [14]. Consequently, any immune responses (e.g. antibody) or drug effective against one isolate will not necessarily be functional against other isolates, maybe not even against different quasispecies in an individual patient. This extreme diversity of the virus is also a major challenge in HCV vaccine development.

**Strategies of HCV vaccine development and clinical trials**

Many different approaches have been tried in HCV vaccine development despite the difficulty raised by the above mentioned extreme diversity of the virus. However, only a few of animal and primate studies have progressed to human trials. Most of these trials have assessed potential therapeutic efficacy of vaccine candidates in patients with HCV infection while a few others tested only in healthy volunteers either towards developing a prophylactic HCV vaccine or as a step for further evaluation in HCV-infected individuals. So far, HCV vaccines have been investigated in human clinical trials.

**Fig. 2.** The genetic organization of the HCV genome and its encoded proteins with indicated molecular weights. Cleavage sites: full circle—signal peptide peptidase, open diamond—signal peptidase, NS2 (full diamond—NS2 and open circle—NS3. ARFP: Alternate reading frame protein. (Adapted from reference 6 and 10)}
studies including mainly the following four forms: recombinant proteins (subunit), synthetic peptides, DNA (plasmid) and viral vectors (virosome). The results of these HCV vaccine approaches in human trials will be reviewed in the following.

1. Recombinant protein vaccines

1.1 Envelope protein vaccines

1.1.1 Prophylactic vaccine

Following successful challenge experiments in chimpanzees, a phase I placebo-controlled, dose-escalation clinical trial in 60 HCV-negative healthy subjects utilized a recombinant E1/E2 heterodimer adjuvanted with adjuvant MF59C (an oil-in-water emulsion) [17]. The vaccine was given at three different dosages on day 0 and weeks 4, 24 and 48. All subjects developed neutralizing antibodies and T-cell lymphocyte proliferation responses to E1/E2, and an inverse response to increasing amounts of antigen was noted. The vaccine was safe and generally well-tolerated at each of the three dosage levels. Neutralizing serum samples had increased affinity levels and displayed more than 2-fold higher specific activity levels to well-characterized epitopes on E1/E2, especially to the hypervariable region 1 of E2 [18]. Moreover, the vaccine also elicits antibodies that cross-neutralize heterologous HCV strains [19]. The authors suggest a larger study to further evaluate efficacy of the vaccine based on recombinant envelope glycoprotein. To date, this is the only published clinical trial of a prophylactic vaccine for HCV.

1.1.2 Therapeutic vaccine

The first candidate therapeutic vaccine for HCV is based on recombinant HCV-E1 protein in alum adjuvant and called InnoVAC-C. The vaccine was administered in 2003 to twenty healthy male volunteers, and thirty-four treatment-naïve chronically HCV infected patients, respectively [20,21]. Following multiple injections over 6 months, the vaccine induced anti-E1 specific antibody and T-cell responses in both groups (50 out of 54). Twenty-four patients underwent a liver biopsy before and after vaccination. In nine of these patients, plasma HCV RNA levels remained unchanged but improvement in liver histology were seen after 17 months. The observed increase in anti-E1 antibody levels correlated with improvement in liver histological scores and decrease in serum alanine transaminase (ALT) levels (a measure of liver inflammation). And twenty-one patients developed a significant de novo E1-specific T-cell response [21]. Moreover, intradermal administration of low dose of E1 could induce E1-specific immune responses and restimulate memory (B and T) cells in patients with resolved HCV infection [22]. Finally, studies on this vaccine progressed to a phase II placebo-controlled, multicenter trial that...
was published in an abstract form in 2008 [23]. The vaccine was evaluated in 122 chronically HCV infected patients who received four courses of six injections over 3 years. Humoral and cellular immune responses to the E1 protein were induced but vaccination did not prevent histological progression of liver disease [23]. Overall, the candidate therapeutic vaccine based on recombinant HCV-E1 was well tolerated. The company Innogenetics in Ghent, Belgium investigating this vaccine eventually abandoned its HCV vaccine program in 2008 and no further work has been published.

1.2 Core protein vaccines

Several B- and T-cell epitopes have been characterized within the HCV core protein and nonstructural protein 3 (NS3) which are highly conserved among various HCV genotypes. A immuno-therapeutic vaccine candidate (GI5005) developed by GlobeImmune Inc. (Louisville, Colorado, USA) is consisting of heat-inactivated recombinant yeast cells (Saccharomyces cervisiae) expressing conserved core–NS3 fusion protein. GI5005 is also a tarmogen (targeted molecular immunogen) designed to elicit antigen-specific host CD4+ and CD8+ T-cell responses for the treatment of chronic HCV infection. Preclinical studies using in vitro and in vivo models demonstrated robust immunogenicity of GI5005. As expected, GI-5005 monotherapy in a phase Ib clinical trial was well tolerated and displayed efficacy in patients with chronic HCV infection [24]. In a Phase II, placebo-controlled trial, GI5005 was evaluated in combination with the standard therapy (PEG-IFN/ribavirin) in more than 250 chronic HCV-1 patients including treatment-naïve and prior non-responders to interferon, compared with the standard therapy alone (details see links 1). Triple therapy resulted in improved early virological responses in all treatment-naïve patients [24] and an increase in sustained virological response (SVR) rates in prior non-responders patients with chronic HCV-1 infection [25]. However, more detailed data are awaited to be peer-reviewed and published.

Another vaccine was developed by using conserved HCV core protein (produced in yeast) combined with an adjuvant composed of saponin, cholesterol and phospholipid, called ISCOMATRIX. Preliminary studies demonstrated that the HCV core ISCOMATRIX vaccine elicited strong CD4+ and CD8+ T-cell responses in monkeys after immunization. The safety, tolerability and immunogenicity of the HCV core ISCOMATRIX vaccine has been evaluated in a phase I placebo-controlled, dose-escalation clinical trial of 30 healthy volunteers [26]. Thirty subjects received three immunizations of HCV core
ISCO MATRIX vaccines or placebo vaccine on days 0, 28 and 56 with three different dosage of 5, 20 or 50 μg HCV core protein with 120 μg ISCOMATRIX adjuvant, respectively. The vaccine was safe and 23 of 24 volunteers who received the vaccine developed a specific antibody response to the core protein in a dose-independent manner. T cell cytokines were detected in 7 of the 8 participants in the highest dose group. However, HCV-specific CD8+ T-cell responses could only be detected in 2 of the 8 participants receiving the highest dose. The same investigators also conducted a phase Ib safety trial of the vaccine in patients with chronic HCV infection who have previously received interferon-based therapy and only mild hepatic damage was observed [26]. Further studies are planned to evaluate this approach as a therapeutic vaccine in HCV-infected patients.

2. Peptide vaccines

Peptide-based vaccines induce HCV-specific T-cell immunity through the direct presentation of vaccine peptide to the T-cell receptor via HLA molecules. Therefore, the major advantage as well as limitation of this approach is that such peptide vaccines are HLA-specific. Consequently, their coverage will be restricted to a subset of the population. Additionally, HCV peptide vaccines to date have included only a handful of peptides and the breadth of the induced T-cell response may be insufficient to control infection.

IC41 is a peptide therapeutic vaccine developed by Intercell AG in Vienna, Austria. It consists of five synthetic peptides from core, NS3 and NS4 proteins that are conserved across HCV genotypes 1 and 2, combined with the T cell adjuvant poly-L-arginine. The peptides contain three CD4+ T-cell and five HLA A2-restricted CD8+ T-cell HCV epitopes. The vaccine has been shown to generate proliferative HCV specific T-cell responses and also IFN-gamma specific responses in enzyme-linked immunosorbent spot (ELISpot) assays in a phase I randomized, placebo-controlled trial of 128 healthy volunteers [27]. A larger phase II double-blind study included 60 HLA-A2 patients with chronic HCV genotype 1 infection, who had previously failed to respond to PEG-IFN/ribavirin. The vaccine was administered subcutaneously to 36 HLA A2 patients and compared with 24 controls who received peptides or adjuvant alone [28]. The vaccine was well tolerated with no serious adverse events. HCV specific T-cell proliferative and IFN-gamma ELISpot assay responses were observed in 67% and 42% of patients, respectively. Three responders had a transient decline in serum HCV RNA (>1 log) associated with the strongest IFN-gamma-secreting T-cell response. A subsequent phase II study
combined IC41 with PEG-IFN/ribavirin therapy in 35 patients with chronic HCV genotype 1 infection. HCV specific T-cell responses were observed in 73% of vaccinated patients and associated with lower HCV-RNA relapse rates \[29\]. Nevertheless, it’s inconclusive regarding vaccine efficacy due to lack of an unvaccinated control group for comparison \[29\]. More recently, weekly or biweekly intradermal (i.d.) IC41 administration (i.e. intensified dosing and/or i.d. injections) was found to induce stronger T-cell responses compared with the original monthly subcutaneous injection approach in healthy subjects \[30\]. This optimized vaccine schedule (i.e. biweekly i.d. IC41 injection) was tested in 50 treatment-naïve patients with chronic HCV genotype 1 infection \[31\]. A significant (p=0.0013) decline of 0.21 log in HCV viral load was observed after 4 months in 44 patients and 24 weeks after the last vaccination the viral load decreased by 0.47 log (p<0.0001) in 34 subjects \[31\]. Intercell AG recently planned for another phase II trial that will combine IC41 with nitazoxanide (a new broad-spectrum antiviral drug) in 60 treatment-naïve patients with chronic HCV genotype 1 infection.

C35-44 peptide is a well known HLA-A2-restricted CTL epitope derived from HCV core region. The peptide could induce CTL activity and humoral responses to both the patients and healthy donors with all the HLA class IA types in Japan \[32\]. Thus, the peptide was evaluated in a phase I dose-escalation study of 26 HCV-positive patients (23 nonresponders to PEG-IFN/ribavirin and three who had refused standard therapy) \[33\]. Twenty-five and 22 patients completed the first and second cycle vaccination (with 6 and 12 vaccine injections), respectively. With a series of six biweekly subcutaneous injections, peptide-specific CD8+ activity was increased in peripheral blood mononuclear cells (PBMCs) from 15 out of 25 patients (measured with ELISpot). Twelve vaccine injections augmented peptide-specific IgG production in plasma from the 15 of 22 patients tested, but not in a dose-dependent mode. Two vaccinated patients had a >1 log decline of HCV RNA. A more than 30% decrease in alanine transaminase and alpha feto-protein was observed in 7 of 24 patients and 3 of 6 patients, respectively. The regimen was well tolerated so further evaluation with a phase II study is recommended \[33\].

A ‘personalized’ peptide vaccine approach was adopted in another phase I clinical trial in Japan. Twelve HCV1b-positive patients who had previously failed PEG-IFN/ribavirin therapy were administered four CD8+ A24 peptides derived from HCV1b in combination with Freund’s adjuvant. Only those peptides that
induced an immune response in each individual were administered biweekly for 14 injections at three different dose settings [34]. After the 7th vaccination, most patients had developed peptide-specific T-cell and antibody responses. After the 14th vaccination, a dose-dependent decrease in serum alanine transaminase and HCV RNA levels was also observed in five and three patients, respectively.

A novel peptide delivery method using autologous monocyte-derived dendritic cells (DCs) was recently developed by Gowans EJ et al. in a phase I dose-escalation clinical trial of DC-based immunotherapy in HCV patients [35]. DCs were loaded and activated ex vivo with HCV-specific HLA A2.1-restricted cytotoxic T-cell epitopes represented in lipopeptides. The cellular vaccine was then administered to six patients chronically infected with HCV who had previously failed PEG-IFN/ribavirin therapy. All patients developed de novo HCV-specific CD8+ T-cell responses (measured by IFN-gamma ELISpot assays). However, epitope spreading might caused T-cell responses induced to additional viral epitopes not presented in the vaccine. T-cell responses were not sustained and no change was observed in viral load, anti-HCV core antibody and circulating cytokines levels.

In addition, a phase I placebo-controlled trial evaluating a virosome-formulated vaccine containing peptides derived from HCV NS3 has recently been completed but no results have been released [41].

Overall, peptide-based HCV vaccines are well tolerated and able to induce peptide-specific T-cell and humoral responses. However, application schedules need to be optimized in order to achieve maximal efficacy since clinical trials show only a few cases had a significant reduction in viral load.

3. DNA vaccines

Substantial research efforts have been made to develop an effective hepatitis C DNA vaccine for HCV infection [36]. As the first DNA-based therapeutic vaccine candidate to reach clinical trial for HCV infection, CICGB-230 is a mixture of a plasmid (pIDKE2) expressing HCV structural antigens (core/E1/E2) with recombinant core protein (Co.120) [38]. CICGB-230 was evaluated in Cuba in a phase I trial of 15 patients with chronic HCV genotype-1 infection who are nonresponders to previous treatment with PEG-IFN/ribavirin therapy. Those patients received monthly intramuscular injections on weeks 0, 4, 8, 12, 16 and 20. The T-cell response to the vaccine components was measured by using ELISpot and proliferation assays. Following the final vaccination, six patients developed de novo
neutralizing antibody responses against heterologous viral pseudoparticles. At 24 weeks after primary immunization, specific T-cell immune responses were observed in 11 patients. Five vaccinated individuals generated de novo cellular immune response against HCV core protein and more patients (7 at the end of treatment) developed cellular immune response against more than one HCV structural antigen during vaccination (p=0.046). Only one patient had a drop in viral load of >1 log. More than 40% of patients had stabilization or improvement in liver histology, which correlated with cellular immune response against more than one HCV antigen (p=0.0053). In addition, vaccination of CICGB-230 in those patients does not impair their immune response to recombinant HBV vaccine [39]. In summary, the vaccine CICGB-230 was well tolerated and thus is a promising candidate for effective therapeutic in individuals with chronic HCV infection.

The second DNA-based HCV vaccine (ChronVac-C) reached a human phase I/IIa clinical trial is a plasmid expressing HCV antigens NS3/4a generated by the company Tripep AB in Stockholm, Sweden [37]. ChronVac-C was delivered intramuscularly by electroporation to enhance the immunogenicity probably through the damage to membranes of the target cells at the site of vaccination. Codon optimization in NS3/4a genes was undertaken to allow effective gene/protein expression and strengthen in vivo T-cell responses. Twelve treatment-naive, genotype-1 HCV infected patients with a low viral load (<800,000 IU/ml) were administered four monthly doses of DNA (three groups: 167, 500 and 1500 μg), respectively. Preliminary results showed 4 of 6 patients who received the higher doses had reductions in viral load >0.5 log lasting for two to more than ten weeks, with corresponding activation of T-cell responses in three of these patients. ChronVac-C was well tolerated, and no vaccine-related severe adverse reactions were observed [37].

4. Viral vector vaccines

Vaccination utilizing live recombinant viral vectors containing target antigens has been proven a very potent strategy for inducing long term protective cellular immune and humoral responses. Therefore, applying this virosome-based approach for the delivery of HCV antigens is a reasonable vaccine choice. Meanwhile, a broader range of viral epitopes may be introduced since the immunogen contained within the vaccine is not HLA restricted. For example, a genetic vaccine based on adenoviral (Ad) vectors has been shown to elicit HCV-specific T-cell responses in vaccinated chimpanzees and suppress acute HCV viremia during primary infection with heterologous virus [15].
Although Ad vectors have shown promise in vaccine trials in animal models, preexisting immunity to common serotypes in humans has limited their use. For example, serotype 5 adenovirus (Ad5) is the most frequently employed Ad vector (from more than 50 human adenovirus subtypes) in immunization studies. Preexisting anti-Ad5 antibodies is abundant in many people throughout the world and thus could significantly weaken Ad5-based vaccine efficacy. To overcome this problem, a better option is to construct other replication-defective Ad vectors of rare serotype like human Ad6 and non-human Ads originating from chimpanzees and simians (which are naturally nonpathogenic to humans). Barnes E et al. constructed such vectors based on Ad6 and chimpanzee Ad3 (ChAd3) harboring HCV1b nonstructural genes NS3-5B [16, 43]. The vaccine vectors are genetically manipulated so that they are nonreplicative and the RNA polymerase activity of the NS5B protein is inactivated to further enhance vaccine safety. 36 healthy volunteers were enrolled and administered intramuscularly in a phase I clinical trial to evaluate the safety and potency of the vaccines singly and in combination as a prime-boost regimen [43]. Both vaccines induced specific CD8+ and CD4+ T-cell responses against multiple HCV proteins in the recipients and these T-cell responses were capable of recognizing heterologous HCV strains (genotypes 1A and 3A). HCV-specific immune response could be sustained for at least a year with boost and vaccination also elicited memory responses. More surprisingly, such vaccination primed broad and sustained immune responses to HCV in healthy human subjects achieved a level consistent with protective immunity. Further studies are planned to confirm prophylactic or therapeutic roles in HCV-infected patients and will enroll 350 subjects (detail see links 2).

Nonreplicative modified vaccinia Ankara (MVA) is a highly attenuated poxvirus strain that has been employed for vaccination against smallpox for long time. MVA is also known as one of the safest vaccine vectors used so far in several vaccine designs for conditions such as HIV, colorectal cancer, TB and melanoma. Hence MVA becomes another attractive recombinant viral vector system for inducing anti-HCV immune responses. A MVA-based therapeutic vaccine (TG4040) expressing HCV NS3-5B proteins has been evaluated for safety and immunogenicity in a phase I open-label, dose-escalation study of 15 chronically infected HCV-1 patients [40]. Those patients received three weekly subcutaneous injections of 10^6, 10^7 and 10^8 plaque-forming units of TG4040, respectively, and were followed for 6 months after the last injection. Vaccination induced HCV-specific cellular immune
responses were observed in 5 of 15 patients (33%). A transient decline in HCV viral load from 0.5–1.4 log was observed in 8 patients in association with a significant CD8+ T-cell response. Overall, TG4040 was well tolerated with no serious adverse events. A phase II trial of TG4040 in combination with standard PEG-IFN/ribavirin treatment is planned [42].

In summary, results of phase I studies on Okairos’ and TG4040 vaccines suggests a great potential for HCV vaccine based on live recombinant viral vectors. And vaccination approaches comprising prime-boost regimen using a recombinant viral vector vaccine alone or together with DNA or subunit vaccine represents a promising strategy for inducing strong immune responses against a broader range of viral epitopes to prevent and/or clear HCV infection.

**Prospective**

As suggested by Roohvand F et al. [6], a successful HCV vaccine is defined different from the conventional one. Prophylactic HCV vaccines shall aim to prevent chronicity of HCV infection rather than to provide de novo sterilizing immunity, whereas therapeutic HCV vaccines aim to improve antiviral efficacy together with the standard therapy.

Besides the above-mentioned HCV vaccine approaches in clinical stages, some other strategies currently in preclinical development include mucosal, plant-based and chimeric virus-like particle (VLP) HCV vaccines that induce strong immune responses in animal models [7]. Among those, VLP vaccines have been successfully developed and used for prevention and control of viral infections like HPV. Such VLP vaccine approach could facilitate delivery of neutralizing antibody and specific T-cell epitopes in a single construct structurally resembling mature virion. Virtually, some essential conformational epitopes could be delivered through this form and hence enhance immunity since recombinant protein or synthetic peptides usually contain only linear antigenic determinants. Garrone P et al. employed such strategy and established a HCV vaccine platform to generate retrovirus-derived virus-like particles (VLPs) pseudotyped with heterologous viral envelope proteins HCV1a E1 and/or E2 [44]. The VLPs are formed like an intact virus with the retroviral Gag protein of Moloney murine leukemia virus (MLV) but are much safer because they lack the viral replication machinery due to a defective genome. Immunization of these VLPs with a prime-boost regimen induced high-titer anti-E1 and/or anti-E2 antibodies, as well as neutralizing antibodies, in mouse and macaque models. These neutralizing antibodies raised against HCV1a could cross-neutralize five other genotypes of
HCV tested (1b, 2a, 2b, 4, and 5). Similarly, broadly neutralizing immune responses against HCV was also achieved in genetically modified mice with vectored measles viruses expressing HCV structural proteins C-E1-E2 and a recombinant envelope protein E2 booster \[^{[45]}\]. These findings together with the clinical trial results obtained earlier with recombinant HCV E1/E2 purified protein as a subunit vaccine \[^{[17-19]}\] suggest a new way to generate a effective multitasking HCV vaccine that induces broadly neutralizing antibodies against multiple viral subtypes and that are still effective after viral mutation. Overall, retrovirus-derived VLPs are formed by the sole expression of the retroviral Gag protein, which can be pseudotyped with a wide range of heterologous envelope proteins of viruses against which no effective vaccine available today, including but not limited to HCV, HIV and RSV. These chimerical VLPs could thus represent a versatile and efficient platform for vaccines aimed at neutralizing antibody induction.

Establishment of \textit{in vitro} HCV culture systems \[^{[4]}\] has also provided the possibility of producing inactivated whole-virus vaccines that eliciting cross-neutralizing antibodies for different HCV genotypes. Those cross-neutralizing antibodies might be used for active and passive immunization strategies. However, the use of such HCV culturing system for developing attenuated HCV viral particles as a vaccine is hindered due to potential dangers associated with it.

Other alternatives include using some currently available novel adjuvants and carriers such as Montanide, CpG ODNs and TLR9 agonists singly or in various combinations, together with different formulas of HCV antigens \[^{[7]}\]. Such strategies may greatly facilitate future studies of HCV vaccines especially those subunit and peptide vaccines.

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Links 1: http://www.globeimmune.com/infectious-disease/gi-5005/

Links 2: http://www.okairos.com