

---

# Progress in Vaccine Development for HCV Infection

---

Ashraf Tabll, Reem El-Shenawy and Yasmine El Abd

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.70649>

---

## Abstract

Hepatitis C virus (HCV) is a blood-transmitted disease that spreads among 3% of the world's population causing seriously increasing mortality rates. The HCV prevalence in Egypt in October 2008 was 14.7% and declined to 6.3% in the survey carried out in October 2015. Nowadays, the new direct-acting antivirals (DAAs) show amazing results especially with regard to HCV genotype 1, but there is still a great necessity to produce a vaccine to avoid this viral infection. Additionally, neutralizing anti-HCV antibodies could be utilized in combination with DAAs empowering their effect. A powerful candidate HCV vaccine should create comprehensively cross-receptive T cells CD4 and CD8 and effectively neutralizing antibodies to successfully clear the virus. The current clinical trials for HCV vaccines comprise synthetic peptides, DNA-based vaccines, or recombinant protein vaccines. Several preclinical vaccine studies are under research including cell culture-derived HCV (HCVcc), HCV-like particles, and recombinant adenoviral vaccines. This mini-review will discuss the prevalence of HCV worldwide and in Egypt. We will present the recent progress in basic research and preclinical and clinical studies for HCV vaccine. Finally, it will present the phenomena of spontaneous clearance of HCV without treatment as a model for study of HCV vaccine development.

**Keywords:** HCV vaccine, cell culture-derived HCV, HCV-like particles, spontaneous clearance, neutralizing antibodies

---

## 1. Introduction

Hepatitis C virus (HCV) infection is the main health problem worldwide and in Egypt. Until now there is no prophylactic or therapeutic vaccine for HCV. The development of a protective vaccine is essential in combating the global HCV epidemic. Understanding the immune response in those who spontaneously resolve HCV infections versus those who develop chronic

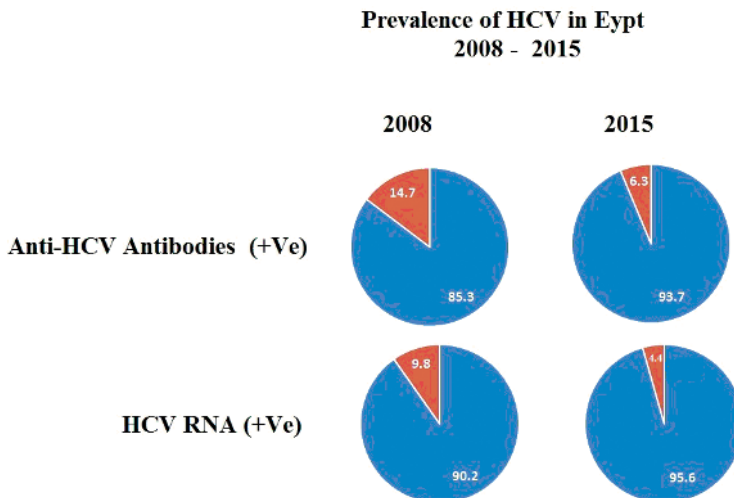
infection is the key to the development of prophylactic or therapeutic vaccine. In this mini review, we will discuss the recent and more promising progress in the HCV vaccine development.

## 2. Prevalence of HCV in the world

HCV is a worldwide predominant pathogen with very high mortality rates [1]. Sub-Saharan Africa accounts for almost one-fifth of worldwide infections; in Southeast Asia, approximately 32.2 million people have chronic HCV infection, and over 6 million infected people are in Latin America [2]. In [3], it is reported that 2.8% people equating more than 185 million are infected by HCV worldwide. Egypt was from the countries with the high prevalence rates (14.7%), followed by Pakistan (4.8%) and China (3.2%). Constant HCV disease is connected with the advancement of liver fibrosis, liver cirrhosis, hepatocellular malignancy, and death [3]. Although the HCV occurrence rate is clearly diminishing in the developing countries, Razavi et al. [4] reported that over the next 20 years the mortality from liver diseases secondary to HCV will keep on rising.

## 3. Prevalence of HCV in Egypt

The 2015 Egypt Health Issues Survey conducted on behalf of the Ministry of Health and Population by El-Zanaty and Associates (<http://www.dhsprogram.com>) showed that the prevalence of HCV antibodies was 6.3% of the tested individuals ( $n = 26,027$ ) in cases with ages 1–59 years, while prevalence of HCV RNA was 4.4%. In 2008, according to the health survey carried out, the prevalence of HCV antibodies was 14.7% (number of examined individuals was 11,126) and that of HCV RNA was 9.8% (as shown in **Figure 1**). Interestingly, a



**Figure 1.** Prevalence of HCV in Egypt according to health survey carried out at 2008 and 2015 in cases aged from 1 to 59 years (male and females).

promising finding of the 2015 survey is that the prevalence of HCV antibodies was 0.7 and HCV RNA was 0.3 among cases with age 10–14, while the percentage of HCV antibodies in cases with ages 50–59 years ( $n = 627$ ) was 30.5–41.9%, and that of HCV RNA was 23.7–27.8%. This means that low prevalence of HCV infection is expected among the future generation. The decline of prevalence of HCV antibodies and HCV RNA is due to single usage of syringes and needles, sterilization of medical, dental and dialysis instruments, regular check-up, testing of blood donors, and also activity of Egyptian National Control Strategy for viral hepatitis.

#### **4. Barriers that limit HCV vaccine development**

HCV is the world's most common blood-borne viral infection for which there is no vaccine [5, 6]. Most acute HCV infections are asymptomatic, and viral persistence is established in the majority of infected subjects. Vaccine development is fundamental to globally eliminate HCV infection through prevention, representing a public health priority. Generally, a good vaccine must induce cellular and humoral immunity, during early phase of viral infection, before the virus gets the opportunity to trigger its numerous immune escape mechanisms [7]. Also a new vaccine should be affordable, safe, not inducing autoimmunity or hypersensitivity, and finally providing long-lasting immunity. Progress in HCV vaccine development is hampered due to the high level of genetic diversity among different HCV strains resulting from the absence of proofreading activity for the NS5B RNA-dependent polymerase [8], which led to the production of genetically distinct but closely related variants within the same genotype designated quasi species [9]. Also, the high viral mutation rate enabled the viral persistence by evading the cellular and humoral immune control [10], either by binding low-density lipoproteins or infecting surrounding cells through cell-to-cell contact mediated by CD81 and Claudin-1 and inducing interfering antibodies by continuous mutation [11]. Also the barriers that challenges vaccine research against HCV is the lack of small and suitable animal models for studying HCV pathogenesis and protective specific immunity. Nowadays, the chimpanzee is the only suitable infectious animal model with lots of ethical and financial obstacles to acquire [12]. Progression to chronic liver disease results from the ineffective weak immune response against the virus. In summary, many barriers occur for HCV vaccine development such as the presence of several HCV genotypes, restricted accessibility of animal models, and the complicated nature of the immunological response to HCV. Neutralizing antibody (Nab) and cellular immune responses to CD4<sup>+</sup> and CD8<sup>+</sup> T cells are essential for HCV clearance [13]. Some reports suggest that the highly changeable, quasi-species' nature of HCV and the continuous emergence of resistant strains are reasons for the HCV resistance attitude. However, the HCV resistance in the presence of circulating antibodies cannot be completely explained by the continuous and rapid acquired viral genetic variability alone.

#### **5. HCV vaccine strategies**

The target of all strategies is to activate a long-lasting T-cell response involving both helper CD4<sup>+</sup> and CD8<sup>+</sup> rather than only adaptive immune response. HCV is vastly mutable, thus developing an effective vaccine is very challenging. In 2013 [14], scientists from Scripps Research Institute

reported that the virus uses HCV E2 envelope glycoprotein as the key protein to invade liver cells. Discovering that E2 binds to the CD81 receptor on the liver cells through a relatively conserved binding region will empower designing of a vaccine which triggers effective antibody responses to various HCV genotypes. Previous studies focused on certain peptide sequences from envelope regions 1 and 2 of HCV as a candidate vaccine. They found that peptide region E1 (aa 315–323) and peptide regions from HCV E2 (aa 412–219) and HCV E2 (aa 517–531) had capability to introduce neutralizing antibodies in mice, rabbits, and goats, while peptide sequence from HCV E2 (aa 430–447) produced nonneutralizing antibodies, which are known interference antibodies [15–22]. Another strategy is to use viral vectors inducing T-cell responses against HCV-infected cells, e.g., adenoviral vectors that have big areas of the HCV genome itself. Early vaccines targeted only genotypes 1a and 1b, accounting for more than 60% of chronic HCV infections worldwide, while subsequent vaccines might target other genotypes by prevalence [23].

Various HCV candidate vaccines were described, comprising synthetic peptides [24], recombinant E1 and E2 proteins [25, 26], recombinant adenoviral and prime-boost strategies with modified vaccinia Ankara (MVA) vaccines or recombinant E1 and E2 glycoproteins [27–30]. However, only few proceeded to phases I and II using recombinant poxvirus [31], DNA vaccines [29, 32], synthetic peptide-based vaccines [33], and MVA vaccines and adenoviral [34, 35]. Recently, Teimourpour et al. [36] successfully cloned structural viral genes in pCDNA3.1 (+) vector and expressed them in eukaryotic expression system facilitating the development of new DNA vaccines against HCV. These candidate vaccines produced robust cross-reactive HCV-specific cellular responses, and HCV viral load was reduced.

On the other hand, plant-based vaccine is a new approach for making an inexpensive and easily producible HCV vaccine. Infecting plants with a genetically engineered tobacco mosaic virus (TMV) produced the hyper variable region 1 (HVR1) peptide fused to the B subunit of cholera toxin CTB. The plant-derived HVR1/CTB reacted with specific antibodies acquired from HCV-infected individuals [37].

## 6. HCV-like particles and cell culture-derived HCV (HCVcc)

Special focus will be drawn on candidate vaccine HCV-like particles and HCVcc, which is expected to boom in the next years. Virus-like particle consists of some of the structural viral proteins. These proteins self-assemble into particles which resemble the virus but lack viral nucleic acid; thus, they are not infectious. Viral-like particles (VLP) are typically more immunogenic because of their highly repetitive and multivalent structure.

### 6.1. HCV-viral like particles (VLP)

HCV VLP vaccine is very promising for the development of a prophylactic vaccine. VLP are vectors for gene delivery that closely resemble the mature HCV. Hence, using a single VLP-based vaccine, neutralizing antibodies and T-cell responses against many epitopes can be induced. Hepatitis B virus (HBV) and human papillomavirus (HPV) have licensed VLP vaccines [38]. Baumert et al. [39] generated HCV-LPs using a recombinant baculovirus containing the complementary DNA for HCV structural proteins in insect cells.

Recently, results of Kumar et al. [40] suggested that the combined regimen of HCV viral-like particles followed by recombinant adenovirus could more effectively inhibit HCV infection, endorsing the novel vaccine strategy.

HCV-LPs were used to immunize four chimpanzees, and all developed HCV-specific T-cell and proliferative lymphocyte responses against core, E1, and E2 proteins. Challenging with infectious HCV, one chimpanzee developed transitory viremia, and the other three displayed higher levels of viremia, but after 10 weeks, their viral levels became immeasurable as reported by Elmowalid et al. [41]. Technique for high-capacity purification of HCV VLPs was defined by Earnest-Silveira et al. [42]. The structural HCV protein coding sequences of genotypes 1a, 1b, 2a, or 3a were coexpressed using a recombinant adenoviral expression system in Huh7 cell line. Using iodixanol ultracentrifugation and Stirred cell ultrafiltration, the structural proteins self-assembled into VLPs which were purified from Huh7 cell lysates. VLPs of the different genotypes are morphologically similar as revealed by electron microscopy. Results showed that it is feasible to produce big quantities of individual HCV genotype VLPs, making this approach an alternative for the manufacture of a quadrivalent mammalian cell-derived HCV VLP vaccine. HCV-specific neutralizing antibodies (Nabs) recognize quaternary structures [43, 44]. The particulate structure of HCV VLPs makes them an attractive vaccine candidate [45–47].

## 6.2. Cell culture-derived HCV (HCVcc)

Kato and Wakita [48] introduced the HCV infection system in cell culture using clone JFH-1, taken from a fulminant HCV-infected Japanese patient. JFH-1 replicates well in hepatic cancer cells and releases infectious virion in the cells' media. Understanding how hosts react to HCV infection and how the viruses escape from host immune reactions was studied using HCVcc systems. Although it is difficult to understand the mechanisms underlying the HCV infection outcomes, innate immune responses seem to have a crucial effect on HCV infection outcomes. Later, robust production of HCVcc particles was obtained by introducing a few specific mutations in JFH-1 structural proteins [49].

Also, Akazawa et al. [50] showed that a protective vaccine can be developed from inactivated HCV particles derived from cultured cells that protected chimeric liver uPA(+/-)-SCID mice against HCV infection. Also, Gottwein and Bukh [51] cultured virus particles constituting the antigen in most antiviral vaccines.

Recently, Yokokawa evaluated neutralizing antibody induction and cellular immune responses following the immunization of a nonhuman primate model with (HCVcc) in [52]. This preclinical study demonstrated that the vaccine included both HCVcc and K3-SPG-induced humoral and cellular immunity in marmosets. Vaccination with this combination resulted in the production of antibodies exhibiting cross-neutralizing activity against multiple HCV genotypes. Based on these findings, the vaccine created in this study represents a promising, potent, and safe prophylactic option against HCV.

Generally, we can conclude the comparison between the two strategies of candidate vaccines of HCVcc and HCV VLP in **Table 1**.

Subject	HCV VLP	HCVcc
Component	Structural core + E1 + E2	Structural and nonstructural
Induction	Induced humoral and cellular immunity	Induced humoral and cellular immunity
Large production	Large production	Not yet
RNA	Lack RNA (noninfectious)	With RNA (infectious particles)
Clinical trail	Not yet	Not yet

**Table 1.** Comparison between candidate vaccines derived from cell culture-derived HCV (HCVcc) and HCV viral-like particles (VLP).

## 7. Clinical trials

Fifty-five studies were conducted on HCV vaccine and were registered for the clinical trial webs, most of them in United States and in Europe (<https://clinicaltrials.gov>). Yet, one study only (ClinicalTrials.gov NCT01436357) revealed encouraging results for population at risk. The study used a replicative defective simian adenoviral vector (ChAd3) and a modified vaccinia Ankara (MVA) vector that encodes HCV genotype 1b proteins, the NS3, NS4, NS5A, and NS5B. It was a placebo-controlled double-blind study with HCV-uninfected male and females aged 18–45 years. In phase I, 68 subjects were enrolled and then an interim analysis of safety data was carried out. Additional 382 volunteers were enrolled in phase II. Very high levels of HCV-specific T cells targeting various HCV antigens were produced giving a persistent memory and effector T cell. Kelly et al. [53] studied the specific HCV immune responses and T-cell cross-reactivity to endogenous virus in chronically HCV-infected genotype 1 patients who were vaccinated using heterologous adenoviral vectors (ChAd3-NSmut and Ad6-NSmut) encoding nonstructural HCV proteins in escalating dose,

ClinicalTrials.gov Identifier	NCT00500747	NCT01436357
Antigen	Envelop (E1 and E2)	Nonstructure (NS3-NS4a-NS4b-NS5a-NS5b)
Composition	Subunit glycoproteins (oil:water adjuvant)	Recombinant virus vectors (Ad and MVA)
Immunity	Neutralizing antibodies and CD4 <sup>+</sup> T cells	CD4 <sup>+</sup> and CD8 T cells
Objective	Neutralize infectivity and prevent persistence	Prevent persistence
Name of company	Chiron (Novartis)	Okairos (GlaxoSmithKline)

**Table 2.** Comparing two candidate HCV vaccines in clinical trial stages.

prime-boost regimen, with and without concomitant pegylated interferon- $\alpha$ /ribavirin therapy. This study concluded that there is a major challenge of overcoming T-cell exhaustion in the context of persistent antigen exposure as the vaccination with potent adenoviral HCV-vectored vaccine was only effective when there is genetic mismatch between immunogen vaccine and endogenous virus.

All trials in **Table 2** are obtained from [www.clinicaltrials.gov](http://www.clinicaltrials.gov) and Ogholikhan and Schwarz [54].

## **8. Studying spontaneous clearance of HCV as a model for developing HCV vaccine**

Studying the immune response in subjects who spontaneously resolve HCV infections is the key to the development of a prophylactic vaccine. Recently, we studied the role of circulating neutralizing antibodies in the spontaneous clearance of HCV in infected blood donors and answered the question of why some anti-HCV-positive donors clear viremia while others do not [19]. Human plasma immunoglobulins targeting HCV E1 region (aa 315–323) and HCV E2 (aa 412–419) and HCV E2, (aa 517–531) in blood donors positive for HCV antibodies were studied. Antibodies targeting HCV E1 region (aa 315–323) and HCV E2 (aa 412–419) and HCV E2 (aa 517–531) possessed cross-neutralizing activity [16, 19].

Spontaneous clearance of HCV is only achieved with early and effective T-cell responses that are entirely efficient with respect to cytolytic capacity, reflected by granzyme and cytokine production [55]. To elaborate the role of cell-mediated immune response in achieving spontaneous clearance, it was documented that patients with hypogammaglobulinemia had the ability to spontaneously clear HCV infection; thus, T-cell responses might be responsible for the protection against HCV [56].

## **9. General conclusion**

HCV is still health problem worldwide and in Egypt, although the prevalence started to decline. HCV vaccine development is urgent as prophylactic and therapeutic agents against new HCV infection. HCV-like particles and cell culture-derived HCV (HCVcc) are the promising candidates for HCV vaccine development. Finally, studying spontaneous clearance of HCV without treatment can be used as a model for HCV vaccine development.

## **Acknowledgements**

This work was partially funded by the project STDF 5041 to Dr. Ashraf Tabll.

## Author details

Ashraf Tabll\*, Reem El-Shenawy and Yasmine El Abd

\*Address all correspondence to: Ashraftabll@yahoo.com

Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Center, Dokki, Giza, Egypt

## References

- [1] Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of Disease: A need to regroup. *Journal of Viral Hepatitis*. 2013;**20**:600-601. DOI: 10.1111/jvh.12123
- [2] Daw MA, Dau AA. Hepatitis C virus in Arab world: A state of concern. *The Scientific World Journal*. 2012;719494. DOI: 10.1100/2012/534173
- [3] Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. *Hepatology*. April 2013;**57**(4):1333-1342. DOI: 10.1002/hep.26141
- [4] Razavi H, ElKhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, et al. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. *Hepatology*. 2013;**57**:2164-2170. DOI: 10.1002/hep.26218
- [5] Rosen HR. Emerging concepts in immunity to hepatitis C virus infection. *Journal of Clinical Investigation*. 2013;**123**:4121-4130. DOI: 10.1172/JCI67714
- [6] Barnes D, Kunitomi M, Vignuzzi M, Saksela K, Andino R. Harnessing endogenous miRNAs to control virus tissue tropism as a strategy for developing attenuated virus vaccines. *Cell Host & Microbe*. 2008;**4**:239-248. DOI: 10.1016/j.chom.2008.08.003
- [7] Ghasemi F, Rostami S, Meshkat Z. Progress in the development of vaccines for hepatitis C virus infection. *World Journal of Gastroenterology*. 2015;**21**:11984-12002. DOI: 10.3748/wjg.v21.i42.11984
- [8] Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: Patterns and determinants. *Nature Reviews Genetics*. 2008;**9**:267-276. DOI: 10.1038/nrg2323
- [9] Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. *Seminars in Liver Diseases*. 1995;**15**:41-63. DOI: 10.1055/s-2007-1007262
- [10] Bowen DG, Walker CM. Mutational escape from CD8+ T cell immunity: HCV evolution, from chimpanzees to man. *Journal of Experimental Medicine*. 2005;**201**:1709-1714. DOI: 10.1084/jem.20050808



- [11] Timpe JM, Stamataki Z, Jennings A, Hu K, Farquhar MJ, Harris HJ, Schwarz A, Desombere I, Roels GL, Balfe P, McKeating JA, Hepatitis C. Virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology*. 2008;**47**:17-24. DOI: 10.1002/hep.21959
- [12] Maily L, Robinet E, Meuleman P, Baumert TF, Zeisel MB. Hepatitis C virus infection and related liver disease: The quest for the best animal model. *Frontiers in Microbiology*. 2013;**4**:213. DOI: 10.3389/fmicb.2013.00212
- [13] Torresi J, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *Journal of Hepatology*. 2011;**54**:1273-1285. DOI: 10.1016/j.jhep.2010.09.040
- [14] Kong L, Giang E, Nieuwma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. Hepatitis C virus E2 envelope glycoprotein core structure. *Science*. 2013;**342**(6162):1090-1094. DOI: 10.1126/science.1243876 PMID: 24288331
- [15] Tabll A, Khalil S, El-Shenawy R, Esmat G, Helmy A, Attallah A, El-Awady M. Establishment of hybrid cell lines producing monoclonal antibodies to a synthetic peptide from E1 region of the hepatitis C virus. *Journal of Immunoassay and Immunochemistry*. 2008;**29**:91-104. DOI: 10.1080/15321810701735161
- [16] Tabll A, El Abd Y, Bader El Din N, El Shenawy R, Abdelhafez T, El Awady M, El-Mohamady H, Viazov S. Establishment of human clones producing neutralizing human monoclonal antibodies to the envelope E1/E2 protein of hepatitis C virus by EBV immortalization of immune CD22+ B cells. *Human Antibodies*. 2013;**22**:55-65. DOI: 10.3233/HAB-140271
- [17] Tabll A, Moustafa R, El Abd Y, Bader El Din N, El-Shenawy R, Yousef H, Hussein M, Dawood R, Omran M, El-Awady M. Mouse monoclonal antibody towards E1 specific epitope blocks viral entry and intracellular viral replication in vitro. *Journal of Immunoassay and Immunochemistry*. 2014;**35**:60-73. DOI: 10.1080/15321819.2013.792831
- [18] Tabll A, Abbas A, El-Kafrawy S, Wahid A. Monoclonal antibodies: Principles and applications of immunodiagnosis and immunotherapy for hepatitis C virus. *World Journal of Hepatology*. 2015;**7**:2369-2383. DOI: 10.4254/wjh.v7.i22.2369
- [19] Tabll A, Salem E, El-Abd Y, El-Shenawy R, Saad A, Abdallah O, Dawood R, El-Awady M. Level of human antibodies targeting HCV E1/E2 peptides and spontaneous clearance of HCV in blood donors. *Clinical Laboratory*. 2016;**62**:1879-1885. DOI: 10.7754/Clin. Lab.2016.160108
- [20] El Abd YS, Tabll AA, Bader El Din NG, Hosny AE, Moustafa RI, El-Shenawy R, Atef K, El-Awady MK. Neutralizing activities of caprine antibodies towards conserved regions of the HCV envelope glycoprotein E2. *Virology Journal*. 2011;**8**:391. DOI: 10.1186/1743-422X-8-391
- [21] El-Awady MK, Tabll A, El-Abd Y, Yousif H, Hegab M, Reda M, El-Shenawy R, Moustafa R, Degheidy N, Bader El Din N. Conserved peptides within the E2 region of hepatitis

- C virus induce humoral and cellular responses in goats. *Virology Journal*. 2009;6(1):66. DOI: 10.1186/1743-422X-6-66
- [22] El-Shenawy R, Tabll A, Bader El Din NG, El Abd Y, Mashaly M, Abdel Malak CA, Dawood R, El-Awady M. Antiviral activity of virocidal peptide derived from NS5A against two different HCV genotypes: An in vitro study. *Journal of Immunoassay and Immunochemistry*. 2015;36:63-79. DOI: 10.1080/15321819.2014.896264
- [23] Kong L, Giang E, Nieuwsma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. Hepatitis C virus E2 envelope glycoprotein core structure. *Science*. 2013;342:1090-1094. DOI: 10.1126/science.1243876
- [24] Kong L, Lee DE, Kadam RU, Liu T, Giang E, Nieuwsma T, Garces F, Tzarum N, Woods Jr. VL, Ward AB, Li S, Wilson IA, Law M. Structural flexibility at a major conserved antibody target on hepatitis C virus E2 antigen. *Proceedings of the National Academy of Sciences of the United States of America*. October 2016. pii: 201609780. PMID: 27791120
- [25] Colombatto P, Brunetto MR, Maina AM, Romagnoli V, Almasio P, Rumi MG, Ascione A, Pinzello G, Mondelli M, Muratori L, Rappuoli R, Rosa D, Houghton M, Abrignani S, Bonino F. *HCV E1E2-MF59* vaccine in chronic hepatitis C patients treated with PEG-IFN $\alpha$ 2a and Ribavirin: A randomized controlled trial. *Journal of Viral Hepatitis*. 2014;21:458-465. DOI: 10.1111/jvh.12163
- [26] Houghton M. Prospects for prophylactic and therapeutic vaccines against the hepatitis C viruses. *Immunological Reviews*. 2011;239:99-108. DOI: 10.1111/j.1600-065X.2010.00977
- [27] Grubor-Bauk B, Yu W, Wijesundara D, Gummow J, Garrod T, Brennan AJ, Voskoboinik I, Gowans EJ. Intradermal delivery of DNA encoding HCV NS3 and perforin elicits robust cell-mediated immunity in mice and pigs. *Gene Therapy*. 2016;23:26-37. DOI: 10.1038/gt.2015.86
- [28] Gummow J, Li Y, Yu W, Garrod T, Wijesundara D, Brennan AJ, Mullick R, Voskoboinik I, Grubor-Bauk B, Gowans EJ. A multiantigenic DNA vaccine that induces broad hepatitis C virus-specific T-cell responses in mice. *Journal of Virology*. 2015;89:7991-8002. DOI: 10.1128/JVI.00803-15
- [29] Sällberg M, Frelin L, Weiland O. DNA vaccine therapy for chronic hepatitis C virus (HCV) infection: Immune control of a moving target. *Expert Opinion on Biological Therapy*. 2009;9:805-815. DOI: 10.1517/14712590902988444
- [30] Chmielewska AM, Naddeo M, Capone S, Ammendola V, Hu K, Meredith L, Verhoye L, Rychlowska M, Rappuoli R, Ulmer JB, Colloca S, Nicosia A, Cortese R, Leroux-Roels G, Balfe P, Bienkowska-Szewczyk K, Meuleman P, McKeating JA, Folgori A. Combined adenovirus vector and hepatitis C virus envelope protein prime-boost regimen elicits T cell and neutralizing antibody immune responses. *Journal of Virology*. 2014;88:5502-5510. DOI: 10.1128/JVI.03574-13
- [31] Habersetzer F, Honnet G, Bain C, Maynard-Muet M, Leroy V, Zarski JP, Feray C, Baumert TF, Bronowicki JP, Doffoël M, Trépo C, Agathon D, Toh ML, Baudin M, Bonnefoy JY,

- Limacher JM, Inchauspé G. A poxvirus vaccine is safe, induces T-cell responses, and decreases viral load in patients with chronic hepatitis C. *Gastroenterology*. 2011;**141**(3):890-899.e1-4. DOI: 10.1053/j.gastro.2011.06.009 PMID: 21699798
- [32] Levander S, Sällberg M, Ahlén G, Frelin L. A non-human hepadnaviral adjuvant for hepatitis C virus-based genetic vaccines. *Vaccine*. 2016;**34**(25):2821-2833. DOI: 10.1016/j.vaccine.2016.04.030
- [33] Klade CS, Schuller E, Boehm T, von Gabain A, Manns MP. Sustained viral load reduction in treatment-naive HCV genotype 1 infected patients after therapeutic peptide vaccination. *Vaccine* 2012;**30**:2943-2950. DOI: 10.1016/j.vaccine.2012.02.070
- [34] Swadling L, Halliday J, Kelly C, Brown A, Capone S, Ansari MA, Bonsall D, Richardson R, Hartnell F, Collier J, Ammendola V, Del Sorbo M, Von Delft A, Traboni C, Hill AV, Colloca S, Nicosia A, Cortese R, Klenerman P, Folgori A, Barnes E. Highly-immunogenic virally-vectored T-cell vaccines cannot overcome subversion of the T-cell response by HCV during chronic infection. *Vaccines (Basel)*. 2016;**4**(3). pii: E27. DOI: 10.3390/vaccines4030027 PMID: 27490575
- [35] Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J, Kurioka A, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Siani L, Traboni C, Hill A, Colloca S, Davis M, Nicosia A, Cortese R, Folgori A, Klenerman P, Barnes E. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Science Translational Medicine*. 2014;**5**(6):261ra153. DOI: 10.1126/scitranslmed.3009185
- [36] Teimourpour R, Tajani AS, Askari VR, Rostami S, Meshkat Z. Designing and development of a DNA vaccine based on structural proteins of hepatitis C virus. *Iranian Journal of Pathology*. 2016;**11**:222-230 PMC5079455
- [37] Nemchinov LG, Liang TJ, Rifaat MM, Mazyad HM, Hadidi A, Keith JM. Development of a plant-derived subunit vaccine candidate against hepatitis C virus. *Archives of Virology*. 2000;**145**:2557-2573 11205105
- [38] Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. *Expert Review of Vaccines*. 2010;**9**:1149-1176. DOI: 10.1586/erv.10.115
- [39] Baumert TF, Vergalla J, Satoi J, Thomson M, Lechmann M, Herion D, Greenberg HB, Ito S, Liang TJ. Hepatitis C virus-like particles synthesized in insect cells as a potential vaccine candidate. *Gastroenterology*. 1999;**117**:1397-1407 10579981
- [40] Kumar A, Das S, Mullick R, Lahiri P, Tatineni R, Goswami D, Bhat P, Torresi J, Gowans EJ, Karande AA, Das S. Immune responses against hepatitis C virus genotype 3a virus-like particles in mice: A novel VLP prime-adenovirus boost strategy. *Vaccine*. 2016;**34**:1115-1125. DOI: 10.1016/j.vaccine.2015.11.061
- [41] Elmowalid GA, Qiao M, Jeong SH, Borg BB, Baumert TF, Sapp RK, Hu Z, Murthy K, Liang TJ. Immunization with hepatitis C virus-like particles results in control of hepatitis

- C virus infection in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:8427-8432. DOI: 10.1073/pnas.0702162104
- [42] Earnest-Silveira L, Chua B, Chin R, Christiansen D, Johnson D, Herrmann S, Ralph SA, Vercauteren K, Mesalam A, Meuleman P, Das S, Boo I, Drummer H, Bock CT, Gowans EJ, Jackson DC, Torresi J. Characterization of a hepatitis C virus-like particle vaccine produced in a human hepatocyte-derived cell line. *Journal of General Virology*. 2016;**97**:1865-1876. DOI: 10.1099/jgv.0.000493
- [43] Keck ZY, Saha A, Xia J, Wang Y, Lau P, Krey T, Rey FA, Fong SK. Mapping a region of hepatitis C virus E2 that is responsible for escape from neutralizing antibodies and a core CD81-binding region that does not tolerate neutralization escape mutations. *Journal of Virology*. 2011;**85**:10451-10463. DOI: 10.1128/JVI.05259-11
- [44] Giang E, Dorner M, Prentoe JC, Dreux M, Evans MJ, Bukh J, Rice CM, Ploss A, Burton DR, Law M. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**:6205-6210. DOI:10.1073/pnas.1114927109
- [45] Elmowalid GA, Qiao M, Jeong SH, Borg BB, Baumert TF, Sapp RK, Hu Z, Murthy K, Liang TJ. Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:8427-8432. DOI: 10.1073/pnas.0702162104
- [46] Chua BY, Johnson D, Tan A, Earnest-Silveira L, Sekiya T, Chin R, Torresi J, Jackson DC. Hepatitis C VLPs delivered to dendritic cells by a TLR2 targeting lipopeptide results in enhanced antibody and cell-mediated responses. *PloS One*. 2012;**7**(10):e47492. DOI: 10.1371/journal.pone.0047492
- [47] Kumar A, Gupta V, Sharma P, Bansal N, Singla V, Arora A. Association of overt diabetes mellitus with the Non-CC but not the CC genotype of Interleukin-28B in hepatitis C virus infected patients. *Journal of Clinical and Translational Hepatology* 2016;**4**:26-31. DOI: 10.14218/JCTH.00040
- [48] Kato T, Wakita T. Production of infectious hepatitis C virus in cell culture. *Uirusu*. 2005;**55**:287-295 16557015
- [49] Delgrange D, Pillez A, Castelain S, Cocquerel L, Rouillé Y, Dubuisson J, Wakita T, Duverlie G, Wychowski C. Robust production of infectious viral particles in Huh-7 cells by introducing mutations in hepatitis C virus structural proteins. *Journal of General Virology* 2007;**88**:2495-2503. DOI: 10.1099/vir.0.82872-0 DOI:10.1099/vir.0.82872-0
- [50] Akazawa D, Moriyama M, Yokokawa H, Omi N, Watanabe N, Date T, Morikawa K, Aizaki H, Ishii K, Kato T, Mochizuki H, Nakamura N, Wakita T. Neutralizing antibodies induced by cell culture-derived hepatitis C virus protect against infection in mice. *Gastroenterology*. 2013;**145**:447-455. DOI: 10.1053/j.gastro.2013.05.007
- [51] Gottwein JM, Bukh J. Viral hepatitis: Cell-culture-derived HCV--a promising vaccine antigen. *Nature Reviews Gastroenterology & Hepatology*. 2013;**10**(9):508-509. DOI: 10.1038/nrgastro.2013.136. PMID: 23897287

- [52] Yokokawa H, Higashino A, Suzuki S, Moriyama M, Nakamura N, Suzuki T, Suzuki R, Ishii K, Kobiyama K, Ishii KJ, Wakita T, Akari H, Kato T. Induction of humoral and cellular immunity by immunisation with HCV particle vaccine in a non-human primate model. *Gut*. 2016;26. pii: gutjnl-2016-312208. DOI: 10.1136/gutjnl-2016-312208
- [53] Kelly C, Swadling L, Capone S, Brown A, Richardson R, Halliday J, von Delft A, Oo Y, Mutimer D, Kurioka A, Hartnell F, Collier J, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Di Marco S, Siani L, Traboni C, Hill AV, Colloca S, Nicosia A, Cortese R, Folgori A, Klenerman P, Barnes E. Chronic hepatitis C viral infection subverts vaccine-induced T-cell immunity in humans. *Hepatology* 2016;63:1455-1470. DOI: 10.1002/hep.28294
- [54] Ogholikhan S, Schwarz KB. Hepatitis vaccines. *Vaccines (Basel)*. 2016;4(1). DOI: 10.3390/vaccines4010006
- [55] Schulze ZJ, Ciuffreda D, Lewis-Ximenez L, Kasprowicz V, Nolan BE, Streeck H, Aneja J, Reyor LL, Allen TM, Lohse AW, McGovern B, Chung RT, Kwok WW, Kim AY, Lauer GM. Broadly directed virus-specific CD4+ T cell responses are primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. *Journal of Experimental Medicine*. 2012;209:61-75. DOI: 10.1084/jem.201003
- [56] Razvi S, Schneider L, Jonas MM, Cunningham-Rundles C. Outcome of intravenous immunoglobulin-transmitted hepatitis C virus infection in primary immunodeficiency. *Journal of Clinical Immunology*. 2001;101:284-288. DOI: 10.1006/clim.2001.5132

