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Immunological scars after cure of hepatitis C virus infection: Long-HepC?

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Hepatitis C virus (HCV) infection provides a unique opportunity to study the effects of spontaneous or treatment-induced viral elimination on the human immune system. Twenty to 50% of patients with acute HCV infection spontaneously clear the virus, which is related to the quality of the individual's immune response, while the chronic infection is associated with an altered and impaired immune response. Direct-acting antiviral agents are now available that provide sustained viral elimination in more than 95% of patients with chronic HCV infection. Viral elimination leads to a decrease in disease sequelae such as cirrhosis and hepatocellular carcinoma, and extrahepatic manifestations also improve. However, some patients may still experience long-term complications, and viral elimination does not protect against HCV reinfection. This review addresses the question of whether the altered and impaired immune response caused by HCV normalizes after viral elimination and if this may affect the long-term clinical course after HCV cure.

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Introduction

Hepatitis C virus (HCV) infections remain a major global health burden, with 1.5 million new HCV infections per year and 58 million chronic HCV infections worldwide. HCV infection either leads to acute, resolving hepatitis or develops into chronic hepatitis with the long-term risk of developing cirrhosis and hepatocellular carcinoma (HCC) [1]. In addition, HCV infection is associated with various extrahepatic manifestations such as fatigue, insulin resistance, or cryoglobulinemia. Chronic HCV infection is associated with an altered and impaired immune response (reviewed in Ref. [2]), which may explain the high rate of chronic infections, but also the risk of reinfection and, in part, the long-term consequences of HCV infection.

Fortunately, interferon (IFN)-free therapies with direct-acting antiviral agents (DAA) have been available for several years. DAAs target either the HCV protease, the HCV protein NS5A, or the HCV polymerase. A combination of an NS5A inhibitor with a protease or polymerase inhibitor is first-line therapy, and if treatment fails, re-treatment with a combination of all three DAA classes is possible. Overall, DAA treatment is well tolerated and now results in a sustained virologic response rate (SVR) of well over 95%, leading to a substantial reduction in liver morbidity and mortality [3]. However, SVR does not protect against reinfection which is of extreme importance, especially for individuals with high risk behaviors [4], and not all sequelae of chronic hepatitis C (CHC) appear to be completely reversible after SVR. Patients with advanced fibrosis or cirrhosis have a residual risk of HCC [5] and impaired quality of life or extrahepatic manifestations are only partially improved or are not reversible in all patients (reviewed in Ref. [3]).

An important question, therefore, arises as to whether the altered immune response normalizes after viral elimination and whether this has an impact on the long-term clinical outcome of patients.

Finally, in contrast to HIV or HBV infections, where complete viral elimination is not or very rarely possible, HCV infection provides a unique model to study the effects of complete viral elimination on changes in immune responses. This may lead to a conceptual understanding of how impaired immune responses can be

restored, which is important not only for chronic infections but also for cancer. In the following sections, we review our (Table 1) and other studies on the immunologic effects of HCV elimination by DAA therapy and the possible consequences for the long-term outcome (Figure 1).

Effects of hepatitis C virus and its elimination on the systemic inflammatory environment

HCV infection results in rapid induction of IFN-stimulated genes (ISG), which elicits a strong type I/III IFN response. Despite this IFN response, most patients with acute HCV infection are unable to completely clear the virus and progress to chronic infection due to various evasion mechanisms. Because of viral persistence, ISG induction and the type I/III IFN response persist and correlate with viral load (reviewed by Ref. [2]). Besides type I/III cytokines also other cytokines and chemokines are altered in patients with acute and chronic HCV infection [6–8]. This inflammatory milieu in HCV infection may also affect the overall immune responses. For example, we have shown that CMV- and EBV-specific T-cell responses in patients with chronic HCV infection have distinct profiles with higher expression of coregulatory receptors (e.g. PD-1 and Tim-3) that could be associated with type I IFN by demonstrating that IFN alpha stimulation induced further upregulation of these markers *in-vitro* [7]. The type I/III milieu may also help to explain clinical features of HCV infection such as chronic fatigue. We have demonstrated that plasma levels of soluble inflammatory mediators (SIM), especially IFN alpha and CXCL-10 but also distinct activation phenotypes of immune cells were associated with neuropsychological parameters [9].

Treatment with DAA and subsequent HCV elimination can potentially normalize the HCV-related inflammatory milieu, for example by downregulation of ISG [10]. However, in the short-term follow-up (FU) of up to 24 weeks after the end of therapy, there does not appear to be complete recovery. We have reported that upregulated SIM levels such as CXCL-10 decreased during and after successful therapy, but not to normal levels in all patients, and that suppressed parameters such as IL-17 did not increase [8]. Similar observations were made in individuals who cleared HCV after liver transplantation [11].

The long duration of HCV exposure of sometimes more than decades in CHC may be one reason for the long-lasting imprinting of the inflammatory milieu after viral elimination. Indeed, a study by Rosenberg et al. showed that gene expression profiles in peripheral blood rapidly normalized to pre-infection levels after spontaneous resolution of acute HCV infection in patients who had thus been viremic for less than six months. However, ISG expression interestingly remained altered over a longer period of time compared with samples from acute dengue virus infection [12]. We have also studied the soluble inflammatory milieu in patients with acute HCV infection [6] who cleared HCV upon DAA therapy in a prospective study [13]. Similar to the study in chronic patients, the majority of SIMs decreased, but not all normalized, and SIMs that were suppressed before DAA treatment remained downregulated [6]. Thus, even after acute HCV infection, the inflammatory response does not seem to completely normalize. However, so far most of the studied patients after both chronic and acute HCV infection were followed up only 12–24 weeks after the end of treatment (EOT). Studies with longer FU times are needed to also analyze the effect after prolonged virus elimination. In addition, the impact of the cytokine milieu after HCV elimination on liver disease progression and extrahepatic manifestations such as chronic fatigue remains to be further determined. Published data suggest a link between systemic inflammation and progression of liver disease and liver cancer in patients with advanced chronic liver disease [14]. We have demonstrated that distinct SIM were associated with the development of HCC after HCV elimination by DAA therapy and this SIM plasma profile of cirrhotic patients who developed HCC after therapy, in contrast to those who remained HCC-free [15].

Likewise, the effects of HCV elimination on the immune response to other pathogens are of interest. The changes in the ISG and inflammatory milieu may influence the heterologous immune response. For example, we have shown that the dominance of HCV and higher serum interferon-γ-induced protein 10 (or CXCL-10) levels was associated with lower quantitative hepatitis B surface antigen in HBV/HCV-coinfected patients [16]. Interestingly, reactivations of hepatitis B virus [17] but also herpes virus [18] infections have been documented after DAA therapy, suggesting that indeed modulations of immune responses after HCV elimination may have clinical implications. The hypothesis as to why reactivation of viral infections occurs may be due to the fact that interferon responses are key elements of antiviral defense, and these are downregulated after HCV eradication [19]. On the other hand, the reduced inflammatory environment may also affect cellular immune responses to other pathogens. We have shown that PD-1 expression on CMV- and EBV-specific $CD8^+$ T-cells decreases after DAA therapy and HCV elimination [7]. Furthermore, direct antigen-specific effects, for example, through T-cell cross-reactivity [20] might also contribute to changes in T-cell responses to other pathogens.

Effects of hepatitis C virus and its elimination on epigenetic imprints

Due to persist antigen stimulation T-cells in chronic infections develop into an exhausted T-cell state.

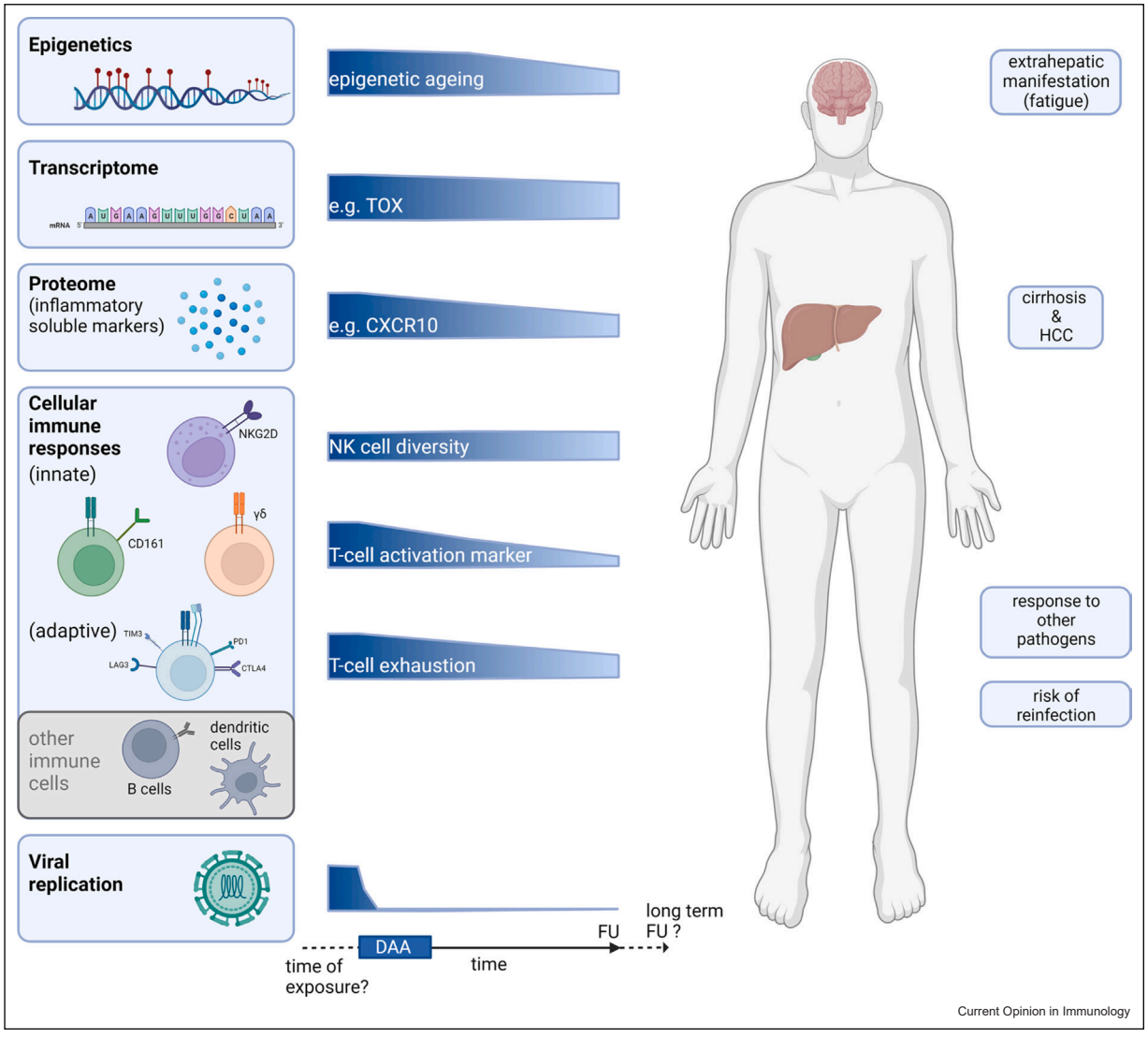
Table 1

Studies conducted in the CRC900 consortium on acute and chronic HCV infection before and after HCV elimination by DAA therapy.

Ref.	Cohorts	Main results
Inflammatory milieu (Proteomics (SIM))		
[7]	CHC patients (n = 76, 69 without, 7 with DAA), healthy controls (n = 78)	In CHC patients, plasma concentrations of cytokines, especially type I/III IFN, were altered. DAA therapy significantly decreased IFNα levels.
[8]	CHC patients (fibrosis and cirrhosis) treated with DAA, FU 12–24 weeks after EOT (n = 28), healthy controls (n = 5), patients with steatosis (n = 20)	The expression patterns of cytokines and chemokines in patients with chronic HCV infection were altered compared with controls. The majority of upregulated analytes decreased during and after DAA therapy, but HCV elimination did not result in complete restoration.
[9]	Patients with chronic liver disease with or without neuropsychiatric symptoms (HCV n = 14, other chronic liver diseases n = 26)	In patients with chronic inflammatory liver disease and neuropsychiatric symptoms, SIM were significantly more upregulated, especially IFNα and CXCL-10, than in patients without symptoms. HCV patients showed a unique pattern of immune alterations.
[11]	Liver transplant recipients (LT): HCV+LT+DAA (n = 25, FU 12–24 weeks after EOT), non-HCV+LT (n = 14), healthy controls (n = 10)	In patients cured of HCV by DAA after liver transplantation, the altered inflammatory milieu did not normalize, nor did the subsets of peripheral immune cells normalize to the levels seen in the absence of HCV recurrence.
[15]	CHC patients with cirrhosis (n = 31, 15 developed HCC, 16 without HCC), healthy controls (n = 10)	Elevated levels of SIMs pre DAA therapy in cirrhosis patients were associated with HCC development post DAA therapy.
[6]	Acute HCV (n = 20, with DAA therapy, FU 24 weeks), chronic HCV (n = 23, with DAA therapy), healthy controls (n = 20)	Profound SIM changes were observed in acute HCV patients. During DAA treatment and FU, the majority of SIM decreased, but not all normalized.
Epigenetics		
[28]	CHC without cirrhosis (n = 22), CHC with cirrhosis (n = 24), CHC with cirrhosis + HCC (n = 8), all treated with DAA therapy, FU up to 96 weeks after EOT	Chronically HCV infected patients showed a significant increase in overall DNA methylation and an increase in epigenetic age acceleration. Elimination of HCV partially reversed the accelerated epigenetic ageing in long-term FU; however, patients with HCC show no change in the acceleration of epigenetic ageing.
Innate cellular immune responses		
[35]	CHC patients (n = 26–42, treated with DAA: FU 12–24 weeks after EOT (n = 26), FU up to 96 weeks after EOT (n = 21)), healthy controls (n = 10–22)	Chronic HCV infection induced a functional imprinting on human NK cells, especially diversity. This imprinting was largely irreversible and remained long after the virus was eliminated.
[37]	CHC patients (n = 23, treated with DAA), healthy controls (n = 9)	Clonality and complexity of the γδ-TCR repertoire were largely comparable to those of healthy controls with more $V\gamma9^+$ T-cells and an increase in Vδ3 clones. Only minor changes in γδ-TCR repertoires were observed after DAA therapy.
[38]	CHC patients (n = 28, treated with DAA), controls with fatty liver disease (n = 20)	In CHC, the frequency, phenotype, and function of MAIT cells in blood were altered. After DAA therapy, MAIT cells were not restored.
[44]	Acute HCV (n = 15 with DAA therapy, FU 24 weeks after EOT), chronic HCV (n = 12, with DAA therapy, FU 48 weeks after EOT), healthy controls (n = 14)	The markedly activated phenotype of unconventional T-cells (e.g. γδ-T cells) during acute HCV infection was reversible after early DAA treatment. However, MAIT cell dysfunction was not restored.
Adaptive cellular immune responses		
[47]	CHC patients without DAA therapy (n = 86), acute HBV (n = 6), acute HCV (n = 13), healthy controls (n = 49)	The study assessed 2B4 expression on HCV-specific $CD8^+$ T-cells during acute and CHC. 2B4 cross-linking could lead to both inhibition and activation of HCV-specific $CD8^+$ T-cell function, depending on expression levels of 2B4 and the intracellular adaptor molecule SAP.
[46]	CHC patients (n = 51, without DAA therapy)	HCV-specific exhausted $CD8^+$ T-cells were characterized by an individualized hierarchy of coregulatory receptor (PD-1, CTLA-4, TIM-3, or 2B4) usage.
[7]	CHC patients (n = 76, 69 without, 7 with DAA), healthy controls (n = 78)	DAA-induced HCV elimination partially reversed the phenotype (higher PD-1 expression) of CMV/EBV-specific $CD8^+$ T-cells in patients with CHC.
[49]	CHC patients (n = 47, n = 28 without cirrhosis, n = 12 with cirrhosis; all treated with DAA, FU 24 weeks after EOT), healthy controls (n = 7)	HCV-specific $CD8^+$ T-cell phenotypes and functional responses were only partially restored after DAA-induced HCV clearance in CHC. The impaired mitochondrial fitness of HCV-specific $CD8^+$ T-cells was not altered after HCV elimination.

DAA – direct-acting antiviral agents; FU – follow-up, EOT – end of treatment, SIM – soluble inflammatory mediators; HCC - Hepatocellular carcinoma.

Figure 1



Schematic illustration of immunological alterations as a result of HCV elimination by DAA therapy in patients with chronic HCV infection *(created with BioRender.com)*.

Recent studies have also shown an impact on the epigenetics of these cells imprinting the exhausted phenotype [21,22]. Chromatin accessibility analysis of HCV specific $CD8^+$ T-cell responses reveals epigenetic signatures of exhaustion that appear to be reversible only to a limited extent after HCV elimination, for example, an epigenetic scar remains in super-enhancers near the *TOX* (thymocyte selection-associated HMG BOX) and *HIF1α* (hypoxia inducible factor-1α) genes [23]. *TOX* is an important regulator of T cell exhaustion [24] and *HIF1α* is a transcriptional regulator of responses to hypoxia and has been linked to T cell activation [25]. Thus, epigenetic scars at loci near critical transcription factors associated with T cell responses may help explain the maintained exhausted immune responses [23].

In addition to showing that the detected epigenetic imprints are not fully reversible after SVR, two groups (Hamdane et al. and Hlady et al.) have detected genome-wide changes in the acetylation of histones (such as H3K4me3, H3K4me1, H3K27ac, and H3K27me3) in hepatocytes that have been linked to increased liver cancer risk [26,27].

In our recent study, we analyzed the epigenetics of immune cells from chronically HCV-infected patients based on DNA methylation. We found a different epigenetic profile in chronically HCV-infected patients compared with healthy controls. These differences could also be reflected by calculating epigenetic age acceleration according to the Horvath clock, patients with CHC

are biologically older. After cessation of DAA therapy (8–12 weeks), epigenetic age acceleration did not improve. Nevertheless, at long-term FU 96 weeks after the end of therapy, we were able to show a significant reduction in age acceleration, which, however, was not completely normalized to the level of healthy controls. Interestingly, patients who developed HCC after DAA therapy showed no improvement in their biological age [28].

The question remains whether the infection with HCV itself or the above-mentioned inflammation is the main trigger for the epigenetic modifications. This needs further investigation, also to discover a treatment that reverses the epigenetic imprints.

Effects of hepatitis C virus and its elimination on cytotoxic cellular innate immune responses

Natural killer (NK) cells are highly enriched in the human liver and contribute as innate lymphocytes to antiviral immune responses. Many studies have suggested an important role for NK cells in the pathogenesis and control of HCV infections (reviewed in Ref. [29]). In chronic HCV infection, NK cells have impaired functions and an altered phenotype [30], which is attributed to the persistent exposure to HCV-induced type I IFN responses [31]. DAA-mediated HCV elimination has been associated decrease in type I IFN responses and, accordingly, normalization of NK cell phenotype and functions [32–34]. However, we have shown that some imprints on NK cells caused by chronic HCV infection do not appear to be completely reversible despite viral elimination. In detail, long term chronic HCV infection increased the inter-individual, but decreased intra-individual NK cell diversity, which was not reversed after DAA therapy and HCV clearance [35]. We are currently investigating the NK cell response in patients with acute HCV infection to determine if a shorter exposure to HCV makes a difference.

Unconventional T-cells, such as $\gamma\delta$ T-cells and Mucosal-associated invariant T (MAIT) cells are unique innate-like T-cells that bridge innate and adaptive immunity. It has been shown that $\gamma\delta$ T-cells are less efficient in producing cytokines and exhibit an activated phenotype in chronic HCV infection [36]. After DAA therapy and clearance of HCV, the $\gamma\delta$ T-cells lose their activated phenotype, but stay dysfunctional [36]. We have investigated if the alteration of the $\gamma\delta$ T-cell compartment is due to skewing in the T-cell receptor repertoire, but this remains stable even after HCV cure with DAA therapy [37].

It was also shown that dysregulation of MAIT cells might play a role in the progression of chronic HCV infection. We and others have shown that these dysfunctions cannot be restored or can only be partially restored after HCV cure achieved by DAA therapy [38–41]. However, the degree of liver inflammation and liver fibrosis may contribute to the degree of dysfunction, loss, and recovery of MAIT cells [40,41]. Alterations in unconventional T-cells in HCV patients and after HCV cure may be important for patients with cirrhosis and especially cirrhosis-related immune dysfunction, as these cells may contribute to immunopathogenesis and immune protection in this setting [42,43].

We investigated whether brief HCV exposure in the setting of acute hepatitis C has an impact on the phenotype and function of unconventional T-cells. We investigated whether a short HCV exposure in the setting of acute hepatitis C has an impact on the phenotype and function of unconventional T-cells. However, while the phenotype of γδ T-cells and MAIT cells normalized their activated phenotype, the frequency and function of MAIT cells remained impaired compared with healthy controls up to 24 weeks after viral elimination by DAA therapy [44].

Effects of hepatitis C virus and its elimination on cellular adaptive immune responses

$CD8^+$ and $CD4^+$ T-cells are the main contributors to the cellular adaptive immune response. A strong and broad virus-specific T-cell response is associated with spontaneous HCV elimination, whereas failure of the T-cell response, either by viral escape or T-cell exhaustion, is associated with the development of chronic infection (reviewed by Refs. [2,45]). Exhaustion of the HCV-specific T-cell response is a hallmark of chronic HCV infection and is characterized by increased coexpression of inhibitory receptors (e.g. PD-1, Tim-3, CTLA-4, and Lag-3, 2B4) [46,47], distinct epigenetic [23], transcriptional [48], and metabolic [49] alterations compared with effector or memory T-cells and impaired functional response (reviewed by Refs. [2,45]). T-cell exhaustion is driven by high and persistent antigen stimulation and in this context, it is a mechanism to prevent an overwhelming immunopathology [50]. The question is whether elimination of the antigen can restore exhausted T-cell responses, which can be addressed using HCV infection as a human model system.

Martin et al.'s study was the first to show an increase in HCV-specific $CD8^+$ T-cells after DAA therapy with a recovery of $CD8^+$ T-cell proliferative capacity [51]. However, further studies, including our own, showed only partial recovery of the phenotype of HCV-specific T-cells and even less restoration of the functional response [49,52,53]. For example, we have shown that mitochondrial dysfunction of exhausted HCV-specific $CD8^+$ T-cells

was not restored in terms of mitochondrial polarization, mitochondrial mass, and reactive oxygen species back to the level of healthy donors [49]. These data indicate that during chronic HCV infection cells develop dysfunctions, which cannot be completely reversed by antigen removal. Recent studies support this finding of a molecular scar also on a transcriptomic level [48]. Furthermore, this concept is supported by the finding that expression of the central transcription factor *TOX*, which controls T-cell exhaustion, decreases after antigen removal but remains higher than in other memory T-cells after recovery from chronic HCV by DAA therapy [24,52]. Importantly, T-cells from chronic HCV infection that were exposed to antigen for a limited time because of the emergence of viral escape mutations were functionally and transcriptionally more similar to memory T-cells from spontaneously resolved HCV infection [52]. In line with this, in one study, HCV-specific T-cell responses were stronger after DAA treatment of acute HCV infection than after DAA treatment of CHC and were similar to those found after spontaneous HCV elimination, except that the breadth of the T-cell response was greater with spontaneous HCV elimination [54].

The before mentioned epigenetic scaring also translates into a molecular scar on the transcriptomic level [23]. This phenomenon of immunological scarring is not only restricted to chronic HCV infections but has also been found in other chronic infections. For example, in chronic lymphocytic choriomeningitis virus (LCMV) infection, similar immunological scars have been identified with functional impairment of virus-specific T cells [55,56]. In addition to this functional impairments, also epigenetic scars have been described to have an impact on memory formation and stay also after clearance of chronic LCMV [23,57].

This highlights, that immunological scarring might be from importance in many chronic viral infections and a possible new therapeutic target.

Most of the data described above were obtained for $CD8^+$ T-cells, but also HCV-specific $CD4^+$ T-cells remained dysfunctional and in very low abundance after HCV cure by DAA therapy for CHC. This study showed comparable phenotypes and functions of $CD4^+$ T-cells resembling protective memory after HCV vaccination and spontaneous HCV elimination [58].

Thus, the current data suggest that the duration of antigen exposure may be relevant for the potential of reversion of the impaired immune response.

In addition, other adaptive immune cells such as regulatory T-cells [59], B-cells [60], or dendritic cells [61] have been studied in the context of chronic HCV infection and cure. However, details will not be further discussed in the review.

Relevance for translation

To further improve the therapy of chronic viral infections such as chronic hepatitis B or HIV (no longer relevant for HCV as being curable), it is important to explore the reasons for immunological scarring and find out how to overcome it. As suggested above, epigenetic changes due to the modulated cytokine environment or the viral infection itself could be the reason for the scarred transcriptome profile and the impaired and metabolically altered T-cell response. To overcome this, correction of the deregulated metabolic pathways underlying T-cell dysfunction, as discussed in the context of hepatitis B virus infection, may represent a novel strategy [62,63]. Another approach could be therapy with epigenetic modifiers such as DNA methyltransferase inhibitors (e.g. 5-aza-2-deoxycytidine or decitabine) or histone deacetylase inhibitors, which in combination with checkpoint inhibition were able to restore an exhausted T-cell response in the LCMV mouse model [64].

The data might also be relevant to understand and also combat long term consequences of HCV infection after cure such as HCC but also extrahepatic manifestations [5]. Chronic fatigue, which is frequently present in patients with CHC is not always reversed after HCV cure [65]. Finally, targeting the impaired immune responses post HCV cure could potentially decrease the risk HCV reinfection.

Another open question that still arises is whether the HCV elimination influences the response to unrelated virus-specific [7] or even tumor associated [66] T-cell responses or if the scaring which can be found in the HCV-specific T-cells after HCV clearance can also be found in immune responses against unrelated antigens.

Finally, the discussion about a scar in the immune response is important for considering controlled human infections models that are considered to study HCV vaccines [67].

Summary and conclusion

In summary, improvement in certain features of the immune system can be observed after successful HCV treatment, but many features of immune exhaustion persist over time after the elimination of the virus. These data could explain the long-term consequences of infections as now discussed in the case of Long-COVID, and understanding the causative mechanisms could contribute to new therapeutic approaches or diagnostics.

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Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. European Association for the Study of the Liver: **EASL recommendations on treatment of hepatitis C: final update of the series**. *J Hepatol* 2020, **73**:1170-1218, https://doi.org/10.1016/j.jhep.2020.08.018

2. Heim MH, Thimme R: **Innate and adaptive immune responses in HCV infections**. *J Hepatol* 2014, **61**:S14-S25, https://doi.org/10.1016/j.jhep.2014.06.035

3. Cornberg M, Manns MP: **The curing regimens of HCV: a SWOT analysis**. *Antivir Ther* 2022, **27**, https://doi.org/10.1177/13596535211072672

4. Hosseini-Hooshyar S, Hajarizadeh B, Bajis S, Law M, Janjua NZ, Fierer DS, *et al*.: **Risk of hepatitis C reinfection following successful therapy among people living with HIV: a global systematic review, meta-analysis, and meta-regression**. *Lancet HIV* 2022, **9**:e414-e427, https://doi.org/10.1016/S2352-3018(22)00077-7

5. • Negro F: **Residual risk of liver disease after hepatitis C virus eradication**. *J Hepatol* 2021, **74**:952-963, https://doi.org/10.1016/j.jhep.2020.11.040.
Comprehensive review about the long-term consequences of chronic hepatitic C after HCV elimination by therapy.

6. • Khera T, Du Y, Todt D, Deterding K, Strunz B, Hardtke S, *et al*.: **Long-lasting imprint in the soluble inflammatory milieu despite early treatment of acute symptomatic hepatitis C**. *J Infect Dis* 2022, **226**:441-452, https://doi.org/10.1093/infdis/jiab048.
Using a proximity extension assay measuring 92 proteins in plasma samples from patients with acute HCV infection, the study showed that the HCV-induced inflammatory milieu did not completely normalize 24 weeks after HCV elimination by DAA therapy.

7. Owusu Sekyere S, Suneetha PV, Hardtke S, Falk CS, Hengst J, Manns MP, *et al*.: **Type I interferon elevates co-regulatory receptor expression on CMV- and EBV-specific CD8 T cells in chronic hepatitis C**. *Front Immunol* 2015, **6**:270, https://doi.org/10.3389/fimmu.2015.00270

8. Hengst J, Falk CS, Schlaphoff V, Deterding K, Manns MP, Cornberg M, *et al*.: **Direct-acting antiviral-induced hepatitis C virus clearance does not completely restore the altered cytokine and chemokine milieu in patients with chronic hepatitis C**. *J Infect Dis* 2016, **214**:1965-1974, https://doi.org/10.1093/infdis/jiw457

9. Aregay A, Dirks M, Schlaphoff V, Owusu Sekyere S, Haag K, Falk CS, *et al*.: **Systemic inflammation and immune cell phenotypes are associated with neuro-psychiatric symptoms in patients with chronic inflammatory liver diseases**. *Liver Int* 2018, **38**:2317-2328, https://doi.org/10.1111/liv.13869

10. Holmes JA, Carlton-Smith C, Kim AY, Dumas EO, Brown J, Gustafson JL, *et al*.: **Dynamic changes in innate immune responses during direct-acting antiviral therapy for HCV infection**. *J Viral Hepat* 2019, **26**:362-372, https://doi.org/10.1111/jvh.13041

11. Aregay A, Engel B, Port K, Vondran FWR, Bremer B, Niehaus C, *et al*.: **Distinct immune imprints of post-liver transplantation hepatitis C persist despite viral clearance**. *Liver Transpl* 2021, **27**:887-899, https://doi.org/10.1002/lt.26031

12. Rosenberg BR, Depla M, Freije CA, Gaucher D, Mazouz S, Boisvert M, *et al*.: **Longitudinal transcriptomic characterization of the immune response to acute hepatitis C virus infection in patients with spontaneous viral clearance**. *PLoS Pathog* 2018, **14**:e1007290, https://doi.org/10.1371/journal.ppat.1007290

13. Deterding K, Spinner CD, Schott E, Welzel TM, Gerken G, Klinker H, *et al*.: **Ledipasvir plus sofosbuvir fixed-dose combination for 6 weeks in patients with acute hepatitis C virus genotype 1 monoinfection (HepNet Acute HCV IV): an open-label, single-arm, phase 2 study**. *Lancet Infect Dis* 2017, **17**:215-222, https://doi.org/10.1016/S1473-3099(16)30408-X

14. Debes JD, van Tilborg M, Groothuismink ZMA, Hansen BE, Schulze zur Wiesch J, von Felden J, *et al*.: **Levels of cytokines in serum associate with development of hepatocellular carcinoma in patients with HCV infection treated with direct-acting antivirals**. *Gastroenterology* 2018, **154**:515-517, https://doi.org/10.1053/j.gastro.2017.10.035

15. Owusu Sekyere S, Port K, Deterding K, Cornberg M, Wedemeyer H: **Inflammatory patterns in plasma associate with hepatocellular carcinoma development in cured hepatitis C cirrhotic patients**. *United Eur Gastroenterol J* 2021, **9**:486-496, https://doi.org/10.1177/2050640620976991

16. Wiegand SB, Jaroszewicz J, Potthoff A, Höner Zu Siederdissen C, Maasoumy B, Deterding K, *et al*.: **Dominance of hepatitis C virus (HCV) is associated with lower quantitative hepatitis B surface antigen and higher serum interferon-γ-induced protein 10 levels in HBV/HCV-coinfected patients**. *Clin Microbiol Infect* 2015, **21**:710e1-9, https://doi.org/10.1016/j.cmi.2015.03.003

17. Mücke MM, Backus LI, Mücke VT, Coppola N, Preda CM, Yeh M-L, *et al*.: **Hepatitis B virus reactivation during direct-acting antiviral therapy for hepatitis C: a systematic review and meta-analysis**. *Lancet Gastroenterol Hepatol* 2018, **3**:172-180, https://doi.org/10.1016/S2468-1253(18)30002-5

18. Perelló MC, Fernández-Carrillo C, Londoño M-C, Arias-Loste T, Hernández-Conde M, Llerena S, *et al*.: **Reactivation of herpesvirus in patients with hepatitis C treated with direct-acting antiviral agents**. *Clin Gastroenterol Hepatol* 2016, **14**:1662-1666.e1, https://doi.org/10.1016/j.cgh.2016.05.016

19. Meissner EG, Wu D, Osinusi A, Bon D, Virtaneva K, Sturdevant D, *et al*.: **Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome**. *J Clin Investig* 2014, **124**:3352-3363, https://doi.org/10.1172/JCI75938

20. Zhang S, Bakshi RK, Suneetha PV, Fytili P, Antunes DA, Vieira GF, *et al*.: **Frequency, private specificity, and cross-reactivity of preexisting hepatitis C virus (HCV)-specific CD8+ T cells in HCV-seronegative individuals: implications for vaccine responses**. *J Virol* 2015, **89**:8304-8317, https://doi.org/10.1128/JVI.00539-15

21. Sen DR, Kaminski J, Barnitz RA, Kurachi M, Gerdemann U, Yates KB, *et al*.: **The epigenetic landscape of T cell exhaustion**. *Science* 2016, **354**:1165-1169, https://doi.org/10.1126/science.aae0491

22. Belk JA, Daniel B, Satpathy AT: **Epigenetic regulation of T cell exhaustion**. *Nat Immunol* 2022, **23**:848-860, https://doi.org/10.1038/s41590-022-01224-z

23. •• Yates KB, Tonnerre P, Martin GE, Gerdemann U, Al Abosy R, Comstock DE, *et al*.: **Epigenetic scars of CD8+ T cell exhaustion persist after cure of chronic infection in humans**. *Nat Immunol* 2021, **22**:1020-1029, https://doi.org/10.1038/s41590-021-00979-1.
Using ATAC-seq, the study showed that the epigenetic state of HCV specific CD8+ T cell exhaustion is largely irreversible, even after HCV elimination by DAA therapy.

24. •• Alfei F, Kanev K, Hofmann M, Wu M, Ghoneim HE, Roelli P, *et al*.: **TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection**. *Nature* 2019, **571**:265-269, https://doi.org/10.1038/s41586-019-1326-9.
Seminal study in LCMV model, but also using human samples from HCV-infected patients, demonstrating that *TOX* is a critical factor in the normal progression of T cell dysfunction and maintenance of exhausted T cells during chronic infections.

25. Doedens AL, Phan AT, Stradner MH, Fujimoto JK, Nguyen JV, Yang E, *et al*.: **Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen**. *Nat Immunol* 2013, **14**:1173-1182, https://doi.org/10.1038/ni.2714

26. Hamdane N, Jühling F, Crouchet E, El Saghire H, Thumann C, Oudot MA, *et al*.: **HCV-induced epigenetic changes associated with liver cancer risk persist after sustained virologic response**. *Gastroenterology* 2019, **156**:2313-2329, https://doi.org/10.1053/j.gastro.2019.02.038

27. Hlady RA, Zhao X, El Khoury LY, Luna A, Pham K, Wu Q, *et al*.: **Interferon drives HCV scarring of the epigenome and creates targetable vulnerabilities following viral clearance**. *Hepatology* 2022, **75**:983-996, https://doi.org/10.1002/hep.32111

28. • Oltmanns C, Liu Z, Mischke J, Tauwaldt J, Mekonnen YA, Urbanek-Quaing M, *et al*.: **Reverse inflammaging: long-term effects of HCV cure on biological age**. *J Hepatol* (1) 2023, **78**:90-98, https://doi.org/10.1016/j.jhep.2022.08.042.
Genome-wide DNA methylation analysis and use of the Horvath clock showed biological age acceleration in HCV patients and partial reversal of age acceleration after HCV elimination by DAA therapy.

29. Lunemann S, Schlaphoff V, Cornberg M, Wedemeyer H: **NK cells in hepatitis C: role in disease susceptibility and therapy**. *Dig Dis* 2012, **30**:48-54, https://doi.org/10.1159/000341680

30. Lunemann S, Malone DFG, Hengst J, Port K, Grabowski J, Deterding K, *et al*.: **Compromised function of natural killer cells in acute and chronic viral hepatitis**. *J Infect Dis* 2014, **209**:1362-1373, https://doi.org/10.1093/infdis/jit561

31. Ahlenstiel G, Titerence RH, Koh C, Edlich B, Feld JJ, Rotman Y, *et al*.: **Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner**. *Gastroenterology* 2010, **138**:325-335e1-2, https://doi.org/10.1053/j.gastro.2009.08.066

32. Serti E, Chepa-Lotrea X, Kim YJ, Keane M, Fryzek N, Liang TJ, *et al*.: **Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function**. *Gastroenterology* 2015, **149**:190-200.e2, https://doi.org/10.1053/j.gastro.2015.03.004

33. Spaan M, van Oord G, Kreefft K, Hou J, Hansen BE, Janssen HLA, *et al*.: **Immunological analysis during interferon-free therapy for chronic hepatitis C virus infection reveals modulation of the natural killer cell compartment**. *J Infect Dis* 2016, **213**:216-223, https://doi.org/10.1093/infdis/jiv391

34. Mele D, Oliviero B, Mantovani S, Ludovisi S, Lombardi A, Genco F, *et al*.: **Adaptive natural killer cell functional recovery in hepatitis C virus cured patients**. *Hepatology* 2021, **73**:79-90, https://doi.org/10.1002/hep.31273

35. Strunz B, Hengst J, Deterding K, Manns MP, Cornberg M, Ljunggren H-G, *et al*.: **Chronic hepatitis C virus infection irreversibly impacts human natural killer cell repertoire diversity**. *Nat Commun* 2018, **9**:2275, https://doi.org/10.1038/s41467-018-04685-9

36. Ghosh A, Mondal RK, Romani S, Bagchi S, Cairo C, Pauza CD, *et al*.: **Persistent gamma delta T-cell dysfunction in chronic HCV infection despite direct-acting antiviral therapy induced cure**. *J Viral Hepat* 2019, **26**:1105-1116, https://doi.org/10.1111/jvh.13121

37. Ravens S, Hengst J, Schlapphoff V, Deterding K, Dhingra A, Schultze-Florey C, *et al*.: **Human γδ T cell receptor repertoires in peripheral blood remain stable despite clearance of persistent hepatitis C virus infection by direct-acting antiviral drug therapy**. *Front Immunol* 2018, **9**:510, https://doi.org/10.3389/fimmu.2018.00510

38. Hengst J, Strunz B, Deterding K, Ljunggren H-G, Leeansyah E, Manns MP, *et al*.: **Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy**. *Eur J Immunol* 2016, **46**:2204-2210, https://doi.org/10.1002/eji.201646447

39. Spaan M, Hullegie SJ, Beudeker BJB, Kreefft K, van Oord GW, Groothuismink ZMA, *et al*.: **Frequencies of circulating MAIT cells are diminished in chronic HCV, HIV and HCV/HIV co-infection and do not recover during therapy**. *PLoS One* 2016, **11**:e0159243, https://doi.org/10.1371/journal.pone.0159243

40. Bolte FJ, O'Keefe AC, Webb LM, Serti E, Rivera E, Liang TJ, *et al*.: **Intra-hepatic depletion of mucosal-associated invariant T cells in hepatitis C virus-induced liver inflammation**. *Gastroenterology* 2017, **153**:1392-1403.e2, https://doi.org/10.1053/j.gastro.2017.07.043

41. Khlaiphuengsin A, Chuaypen N, Sodsai P, Reantragoon R, Han WM, Avihingsanon A, *et al*.: **Successful direct-acting antiviral therapy improves circulating mucosal-associated invariant T cells in patients with chronic HCV infection**. *PLoS One* 2020, **15**:e0244112, https://doi.org/10.1371/journal.pone.0244112

42. Niehaus CE, Strunz B, Cornillet M, Falk CS, Schnieders A, Maasoumy B, *et al*.: **MAIT cells are enriched and highly functional in ascites of patients with decompensated liver cirrhosis**. *Hepatology* 2020, **72**:1378-1393, https://doi.org/10.1002/hep.31153

43. Mehta H, Lett MJ, Klenerman P, Filipowicz Sinnreich M: **MAIT cells in liver inflammation and fibrosis**. *Semin Immunopathol* 2022, **44**:429-444, https://doi.org/10.1007/s00281-022-00949-1

44. Du Y, Khera T, Strunz B, Deterding K, Todt D, Woller N, *et al*.: **Imprint of unconventional T-cell response in acute hepatitis C persists despite successful early antiviral treatment**. *Eur J Immunol* 2022, **52**:472-483, https://doi.org/10.1002/eji.202149457

45. Rehermann B: **Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells**. *Nat Med* 2013, **19**:859-868, https://doi.org/10.1038/nm.3251

46. Owusu Sekyere S, Suneetha PV, Kraft ARM, Zhang S, Dietz J, Sarrazin C, *et al*.: **A heterogeneous hierarchy of co-regulatory receptors regulates exhaustion of HCV-specific CD8 T cells in patients with chronic hepatitis C**. *J Hepatol* 2015, **62**:31-40, https://doi.org/10.1016/j.jhep.2014.08.008

47. Schlaphoff V, Lunemann S, Suneetha PV, Jaroszewicz J, Grabowski J, Dietz J, *et al*.: **Dual function of the NK cell receptor 2B4 (CD244) in the regulation of HCV-specific CD8+ T cells**.

PLoS Pathog 2011, **7**:e1002045, https://doi.org/10.1371/journal.ppat.1002045

48. •• Hensel N, Gu Z, Sagar, Wieland D, Jechow K, Kemming J, *et al*.: **Memory-like HCV-specific CD8+ T cells retain a molecular scar after cure of chronic HCV infection**. *Nat Immunol* 2021, **22**:229-239, https://doi.org/10.1038/s41590-020-00817-w.
Using single-cell transcriptomics, the study identified a molecular signature of HCV-specific CD8+ T cell exhaustion that is maintained as a chronic scar even after HCV elimination by DAA therapy.

49. Aregay A, Owusu Sekyere S, Deterding K, Port K, Dietz J, Berkowski C, *et al*.: **Elimination of hepatitis C virus has limited impact on the functional and mitochondrial impairment of HCV-specific CD8+ T cell responses**. *J Hepatol* 2019, **71**:889-899, https://doi.org/10.1016/j.jhep.2019.06.025

50. Cornberg M, Kenney LL, Chen AT, Waggoner SN, Kim S-K, Dienes HP, *et al*.: **Clonal exhaustion as a mechanism to protect against severe immunopathology and death from an overwhelming CD8 T cell response**. *Front Immunol* 2013, **4**:475, https://doi.org/10.3389/fimmu.2013.00475

51. Martin B, Hennecke N, Lohmann V, Kayser A, Neumann-Haefelin C, Kukolj G, *et al*.: **Restoration of HCV-specific CD8+ T cell function by interferon-free therapy**. *J Hepatol* 2014, **61**:538-543, https://doi.org/10.1016/j.jhep.2014.05.043

52. •• Tonnerre P, Wolski D, Subudhi S, Aljabban J, Hoogeveen RC, Damasio M, *et al*.: **Differentiation of exhausted CD8+ T cells after termination of chronic antigen stimulation stops short of achieving functional T cell memory**. *Nat Immunol* 2021, **22**:1030-1041, https://doi.org/10.1038/s41590-021-00982-6.
Important study on HCV specific CD8+ T cell responses that suggest that the duration of T cell stimulation impacts exhaustion recovery, with antigen removal after long-term exhaustion being insufficient for the development of functional T cell memory.

53. Llorens-Revull M, Costafreda MI, Rico A, Guerrero-Murillo M, Soria ME, Píriz-Ruzo S, *et al*.: **Partial restoration of immune response in hepatitis C patients after viral clearance by direct-acting antiviral therapy**. *PLoS One* 2021, **16**:e0254243, https://doi.org/10.1371/journal.pone.0254243

54. Casey JL, Dore GJ, Grebely J, Matthews GV, Cherepanov V, Martinello M, *et al*.: **Hepatitis C virus-specific immune responses following direct-acting antivirals administered during recent hepatitis C virus infection**. *J Viral Hepat* (1) 2023, **30**:64-72, https://doi.org/10.1111/jvh.13761

55. Utzschneider DT, Legat A, Fuertes Marraco SA, Carrié L, Luescher I, Speiser DE, *et al*.: **T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion**. *Nat Immunol* 2013, **14**:603-610, https://doi.org/10.1038/ni.2606

56. Utzschneider DT, Gabriel SS, Chisanga D, Gloury R, Gubser PM, Vasanthakumar A, *et al*.: **Early precursor T cells establish and propagate T cell exhaustion in chronic infection**. *Nat Immunol* 2020, **21**:1256-1266, https://doi.org/10.1038/s41590-020-0760-z

57. Abdel-Hakeem MS, Manne S, Beltra J-C, Stelekati E, Chen Z, Nzingha K, *et al*.: **Epigenetic scarring of exhausted T cells hinders memory differentiation upon eliminating chronic antigenic stimulation**. *Nat Immunol* 2021, **22**:1008-1019, https://doi.org/10.1038/s41590-021-00975-5

58. Hartnell F, Esposito I, Swadling L, Brown A, Phetsouphanh C, de Lara C, *et al*.: **Characterizing hepatitis C virus-specific CD4+ T cells following viral-vectored vaccination, directly acting antivirals, and spontaneous viral cure**. *Hepatology* 2020, **72**:1541-1555, https://doi.org/10.1002/hep.31160

59. Langhans B, Nischalke HD, Krämer B, Hausen A, Dold L, van Heteren P, *et al*.: **Increased peripheral CD4+ regulatory T cells persist after successful direct-acting antiviral treatment of chronic hepatitis C**. *J Hepatol* 2017, **66**:888-896, https://doi.org/10.1016/j.jhep.2016.12.019

60. Nishio A, Hasan S, Park H, Park N, Salas JH, Salinas E, *et al*.: **Serum neutralization activity declines but memory B cells persist after cure of chronic hepatitis C**. *Nat Commun* 2022, **13**:5446, https://doi.org/10.1038/s41467-022-33035-z

61. Auffermann-Gretzinger S, Keeffe EB, Levy S: **Impaired dendritic cell maturation in patients with chronic, but not resolved, hepatitis C virus infection**. *Blood* 2001, **97**:3171-3176, https://doi.org/10.1182/blood.v97.10.3171

62. Pallett LJ, Gill US, Quaglia A, Sinclair LV, Jover-Cobos M, Schurich A, *et al*.: **Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells**. *Nat Med* 2015, **21**:591-600, https://doi.org/10.1038/nm.3856

63. Fisicaro P, Barili V, Montanini B, Acerbi G, Ferracin M, Guerrieri F, *et al*.: **Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B**. *Nat Med* 2017, **23**:327-336, https://doi.org/10.1038/nm.4275

64. Ghoneim HE, Fan Y, Moustaki A, Abdelsamed HA, Dash P, Dogra P, *et al*.: **De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation**. *Cell* 2017, **170**:142-157.e19, https://doi.org/10.1016/j.cell.2017.06.007

65. Ohlendorf V, Schäfer A, Christensen S, Heyne R, Naumann U, Link R, *et al*.: **Only partial improvement in health-related quality of life after treatment of chronic hepatitis C virus infection with direct acting antivirals in a real-world setting-results from the German Hepatitis C-Registry (DHC-R)**. *J Viral Hepat* 2021, **28**:1206-1218, https://doi.org/10.1111/jvh.13546

66. Owusu Sekyere S, Schlevogt B, Mettke F, Kabbani M, Deterding K, Wirth TC, *et al*.: **HCC immune surveillance and antiviral therapy of hepatitis C virus infection**. *Liver Cancer* 2019, **8**:41-65, https://doi.org/10.1159/000490360

67. Liang TJ, Feld JJ, Cox AL, Rice CM: **Controlled Human Infection Model — fast track to HCV vaccine**. *N Engl J Med* 2021, **385**:1235-1240, https://doi.org/10.1056/NEJMsb2109093