Review

Immune Reconstitution After HCV Clearance With Direct Antiviral Agents: Potential Consequences for Patients With HCC?

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Abstract: Recent introduction of all-oral direct-acting antiviral (DAA) treatment has revolutionized care of patients with chronic hepatitis C virus infection. Because patients with different liver disease stages have been treated with great success including those awaiting liver transplantation, therapy has been extended to patients with hepatocellular carcinoma as well. From observational studies among compensated cirrhotic hepatitis C patients treated with interferon-containing regimens, it would have been expected that the rate of hepatocellular carcinoma occurrence is markedly decreased after a sustained virological response. However, recently 2 studies have been published reporting markedly increased rates of tumor recurrence and occurrence after viral clearance with DAA agents. Over the last decades, it has been established that chronic antigen stimulation during persistent infection with hepatitis C virus is associated with continuous activation and impaired function of several immune cell populations, such as natural killer cells and virus-specific T cells. This review therefore focuses on recent studies evaluating the restoration of adaptive and innate immune cell populations after DAA therapy in patients with chronic hepatitis C virus infection in the context of the immune responses in hepatocarcinogenesis.

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EARLY RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER DIRECT-ACTING ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

Chronic infection with the hepatitis C virus (HCV) is a leading cause of end-stage liver disease, hepatocellular carcinoma (HCC) and liver-related death in the Western world.[1] A major driving factor in the development of HCC is chronic liver inflammation and subsequent cirrhosis. Once liver cirrhosis is present there is a 1% to 5% annual risk of HCC development.[2] HCC is the sixth most common cancer and the third most common cause of cancer-related death worldwide.[3] Thus, the majority of patients present with locally advanced or metastatic disease with several tumors in the liver. Since a potentially curative resection is only feasible in patients with mild cirrhosis, an orthotopic liver transplantation (OLT) provides the only treatment option for patients with end-stage disease by simultaneously combining a complete removal of the tumor (with potential micrometastases) with replacing the cirrhotic liver as a predisposing factor.[2]

HCV reinfection is universal in OLT recipients and often results in accelerated progression of fibrosis in the grafted liver. It is therefore compelling in the era of direct-acting antiviral (DAA) therapy that chronic HCV patients awaiting liver transplantation due to liver failure should immediately be treated to prevent graft infection.[4] However, these recommendations are less explicit for patients with HCC and chronic HCV infection. Most of the hesitation stems from 2 recent studies reporting increased rates of HCC recurrence in patients who cleared HCV after interferon-free DAA therapy. In the first retrospective cohort study from Italy, chronic HCV-infected cirrhotic patients either without a history of HCC (n = 285) or with a complete response to prior treatment of HCC (n = 59), were followed up for up to 24 weeks after DAA therapy. While a de novo occurrence of HCC was detected in 26 of 285 (7.6%) patients, an alarmingly high rate of recurrence of previous successfully treated HCC was reported in 17 (29%) of 59 patients.[5] In a second

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study from Spain, Reig and colleagues[6] followed up 58 patients with a prior history of HCC and complete response to resection, ablation or chemoembolization who underwent interferon-free DAA therapy. After a median follow up of 5.7 months, 16 (28%) of 58 patients developed a HCC recurrence. which again is an unexpectedly high rate of tumor recurrence. In contrast to these 2 studies, a retrospective analysis of the Agence Nationale de Recherche sur le Sida (ANRS) register in France that prospectively includes patients with HCV infection, does not suggest an increased risk of HCC recurrence after DAA therapy.[7] However, in contrary to both previous studies from Italy and Spain, the ANRS register also includes patients who received an OLT. Notably, focusing only on those patients without OLT in the ANRS register, 189 HCV patients could be identified with a history of HCC and complete response to local treatment. After receiving interferon-free DAA therapy, 24 (12.4%) of 189 patients developed HCC recurrence; although the result represents a slightly increased tumor recurrence rate, there was a better outcome compared with the Italian and Spanish studies.

Despite methodological concerns regarding all 3 studies that arise from combining heterogeneous groups of patients who receive different treatments, including palliative (transarterial chemoembolization) and curative (ablation, surgery and liver transplantation) approaches for HCC, along with limited sample sizes and variable time of follow-up after DAA therapy (especially differences in the time frame between HCC treatment and HCV therapy), these conflicting reports can only be compared with observational studies among compensated cirrhotic hepatitis C patients treated by IFN-containing regimens in the recent era of HCV therapy. In these patients, the rate of de novo HCC occurrence was markedly decreased after a sustained virological response (SVR), but not abolished with an incidence of HCC ranging between 1% and 2% per year after viral eradication.[8] Furthermore, in a meta-analysis of 6 randomized controlled trials, including a total of 374 HCC patients, IFN treatment of 201 chronic HCV patients led to better survival and lower HCC recurrence rates compared with 173 control patients without treatment.[9] Although the explanation for different outcomes in IFN-containing regimens is unknown, a reason could be selection bias in studies concentrating on DAA treatment, because they include patients with severely impaired liver function who would not have qualified for the previous IFN treatment and would therefore have ended up in the respective control group with a worse outcome. Because there are substantial differences especially in the time frame between HCC treatment and HCV therapy among all of these studies, there might also be differences in the probability of persistent tumor cells. Another hypothesis goes along the line that the rapid decrease of viral load in the liver after DAA treatment quickly diminishes the endogenous IFN response to the virus in the liver and thus may cause an imbalanced antitumor immune response.[10] This phenomenon will not occur in patients on IFN treatment where the immune system has more time to balance itself until the end of treatment, considering the rather slow antiviral effect of IFN.

Prospective studies are needed to finally assess whether DAA treatment leads to an increased rate of HCC recurrence. To understand how HCC recurrence may be influenced by the elimination of HCV, a better understanding of how DAA treatment alters HCV-specific and nonspecific immune responses and the microenvironment in the liver is needed. In the following sections, we will discuss the current knowledge on immune responses against HCV and potential influences on hepatocarcinogenesis.

IMPAIREMENT OF ADAPATIVE AND INNATE IMMUNE CELLS IN PATIENTS WITH CHRONIC HCV INFECTION

Immune responses of patients with chronic HCV infection have been studied extensively over the past 3 decades to understand the immunological mechanisms of persistent infection and to search for protective immunity. Because several excellent reviews have covered the whole field of adaptive and innate immune responses, we will only summarize the major points here that characterize the immunological baseline status of chronic HCV patients before start of interferon-free DAA therapy.

Innate immune responses are the first-line of defense against viral infections and IFNs are a main driver of immune cell activation, including cellular components of innate immunity. In patients with chronic HCV infection, induction of IFN-stimulated genes (ISGs) varies considerably between individuals. Half of the patients have hundreds of type I or III ISGs constitutively expressed at high levels in the liver, whereas the other half experience no detectable induction.[11] Induction of the endogenous IFN system in the liver apparently has little antiviral efficacy, because HCV persists for decades despite the expression of various ISGs. Notably, patients with an activated endogenous IFN system have even been reported to be poor responders to IFN-based therapies.[12] However, half of the patients undergoing OLT due to the consequences of HCV infection seem to tolerate the withdrawal of immunosuppressive drugs and spontaneously tolerate the liver graft.[13] Tolerance was associated with intrahepatic overexpression of type I IFN and immunoregulatory genes and with an expansion of exhausted HCV-specific circulating CD8+ T cells.[13] This indicates that HCV persistence exerts immunoregulatory effects that can even contribute to the restraining of alloimmune responses against a liver graft.

Natural killer (NK) cells are not only the major effector cells of the innate immune response but also play a distinct role in chronic HCV infection. NK cells from chronic HCV-infected patients show an activated phenotype with a higher expression of several activating receptors, such as NKp30, NKp44, NKp46, NKG2C, NKG2D, and CD122.[14] However, NK cell activation does not include all effector functions. Whereas correlates of cytotoxicity such as degranulation and TNF-related apoptosis-inducing ligand (TRAIL) expression are increased in chronic HCV infection, IFN-γ and TNF-α production are decreased. This polarized cytotoxic function with an impairment of anti-viral cytokine production has been linked to chronic exposure to cytokines, especially IFN-α.[14]

Adaptive, antigen-specific immune responses are functionally impaired in patients with chronic HCV infection. Neutralizing antibodies that target E1E2 are readily detected in the serum of chronically infected patients without any apparent impact on disease outcome.[15] Several mechanisms have been described that may contribute to evasion of sterilizing Ab-mediated clearance of HCV. These include sequence changes, decoy epitopes, epitope masking, lipid shielding, and induction of interfering antibodies.[15] Virus-specific T cell responses are skewed in the chronic phase of HCV infection, due to viral escape and functional exhaustion of virus-specific CD4+ and CD8+ T cells. Consequently, remaining antigen-specific T cells show increased expression of inhibitory molecules such as PD-1, cytotoxic T lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin domain–containing molecule 3 and 2B4 (CD244), with the corresponding ligands being upregulated in the inflamed liver.[16]

Another immunological player entered the field more recently. Mucosal-associated invariant T (MAIT) cells are an abundant innate-like T cell subset that is defined by the invariant T cell receptor V-alpha chain Vα7.2-Jα33. MAIT cells represent about 5% of peripheral T cells and can represent up to 50% of intrahepatic T cells. In terms of effector function, MAIT cells are able to secret multiple cytokines such as IL-17, IFN-γ or TNF-α and release cytotoxic enzymes.[17] In chronic HCV infection, the frequency of peripheral MAIT cells is decreased compared with healthy controls and MAIT cells show signs of chronic immune activation and resulting immune exhaustion.[18,19] Activation markers, such as HLA-DR or CD38, are upregulated on MAIT cells of chronic HCV-infected patients and, in parallel, expression of exhaustion markers, such as PD-1, CTLA-4, and Tim-3 are increased.[18] In addition, MAIT cells are impaired in their MR-1-dependent effector functions. In contrast to healthy controls, MAIT cells from chronic HCV-infected patients show decreased production of cytokines such as IL-17, IFN-γ, or TNF and respond with reduced degranulation after stimulation with *Escherichia coli*.[20]

Peripheral blood monocytes are in general attracted to the site of infection via chemokines, such as CCL2 or CCL1 in response to danger and toll-like receptor signals and differentiate into different macrophage subtypes.[21] The majority of the body's macrophages reside in the liver.[22] In the liver, Kupffer cells (KC) make up most of the resident macrophage population. KC located in liver sinusoids and are specialized to perform phagocytic functions, thereby eliminating protein complexes, small particles and apoptotic cells from the portal blood flow.[23] In patients with chronic HCV infection, compared with healthy controls, KC are increased in numbers in the liver and exhibit an activated phenotype with increased expression of activation markers such as CD163 and CD33.[24,25] In addition, KC release IL-1β and IL-18 in response to HCV exposure in vitro.[26] Consequently, coexpression of IL-1β and CD68 has been observed using immunofluorescence on liver biopsies from patients with chronic hepatitis C. Likewise, elevated IL-1β levels have been detected in HCV patient serum compared with healthy individuals.[27] Peripheral monocytes from patients with chronic HCV infection, in contrast to KC, show an attenuated capacity to produce TNF-α and also have reduced indirect IL-18 and NK cell–mediated antiviral effects.[28]

RESTORATION OF IMMUNE CELL FUNCTION AFTER ELIMINATION OF HCV BY INTERFERON-FREE DAA THERAPY

Recent introduction of all-oral DAA treatment has revolutionized the care of patients with chronic HCV infection because they achieve sustained response rates above 90%.[29] DAA agents fall into four classes defined by steps in the life cycle of HCV that they block—namely, NS3/4A protease inhibitors (eg, paritaprevir), NS5B nucleoside polymerase inhibitors (eg, sofosbuvir), NS5B nonnucleoside polymerase inhibitors (eg, dasabuvir), and NS5A inhibitors (eg, ledipasvir, daclatasvir).[30] These drugs are administered in combinations chosen on the basis of viral genotype and residual liver function.[31,32] These new treatment regimens are not only exciting from a clinical point of view, but also from an immunological perspective. A key question is whether effective elimination of HCV is able to restore the impaired adaptive and innate immune responses (Figure 1).

Looking at the innate immune response, treatment of chronic HCV genotype 1 patients for 12 weeks with a combination of sofosbuvir and ribavirin reveals that viral clearance is accompanied by rapid downregulation of ISGs in liver and blood, regardless of treatment outcome. However, patients who achieved an SVR had unexpectedly higher intrahepatic expression of ISGs at the end of treatment compared to patients who relapsed. Analysis of paired pretreatment and end of treatment liver biopsies from patients that achieved SVR have shown that viral clearance is accompanied by decreased expression of type II and several type III IFNs in parallel with ISGs. However, expression of type I IFNs unexpectedly increased (IFNA2) at the end of treatment.[10] This suggests that the higher ISG expression observed in paired liver biopsies from patients who achieved SVR could be driven by an enhanced end of treatment type I IFN response, and that this in turn supports complete elimination of residual virus. Therefore, faster reestablishment of IFN homeostasis in the liver after chronic IFN stimulation may promote achievement of SVR.

Focusing on antigen-specific T cells, Martin et al were the first to describe restoration of proliferative HCV-specific CD8+ T cells in the majority of patients with a SVR after 12 weeks of DAA therapy. Most important, the frequency of Flu-specific and cytomegalovirus-specific CD8+ T cells did not increase significantly during treatment, indicating that the increase is not a result of unspecific T cell proliferation mediated, for example, by ribavirin. Furthermore, cocultures with HCV replicon cells revealed a higher antiviral efficacy of HCV-specific CD8+ T cells after successful DAA treatment, suggesting that HCV removal by DAA restores both the proliferative capacity and CD8+ T cell function.[33] Consistent with restored T cell function, a decrease in PD-1 expression on HCV-specific CD8+ T cells indicates a recovery from T cell exhaustion.[34] However, after recovery intrahepatic HCV-specific CD8+ T cells failed to prevent HCV infection as well as virus persistence in chimpanzees after a new virus challenge 2 years after cure of chronic HCV infection with DAAs.[35]

Another interesting observation after initiation of DAA therapy is a rapid decrease in HCV RNA associated with a decline of IP-10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1 beta, and IL-18 levels in the serum at varying rates.[36] These changes in the level of inflammatory cytokines coincide with a decrease in the activation of intrahepatic and blood NK cells. This effect is followed by restoration of a normal NK cell phenotype and function by week 8 in patients with undetectable viremia and is maintained until the end of treatment.[37,38]

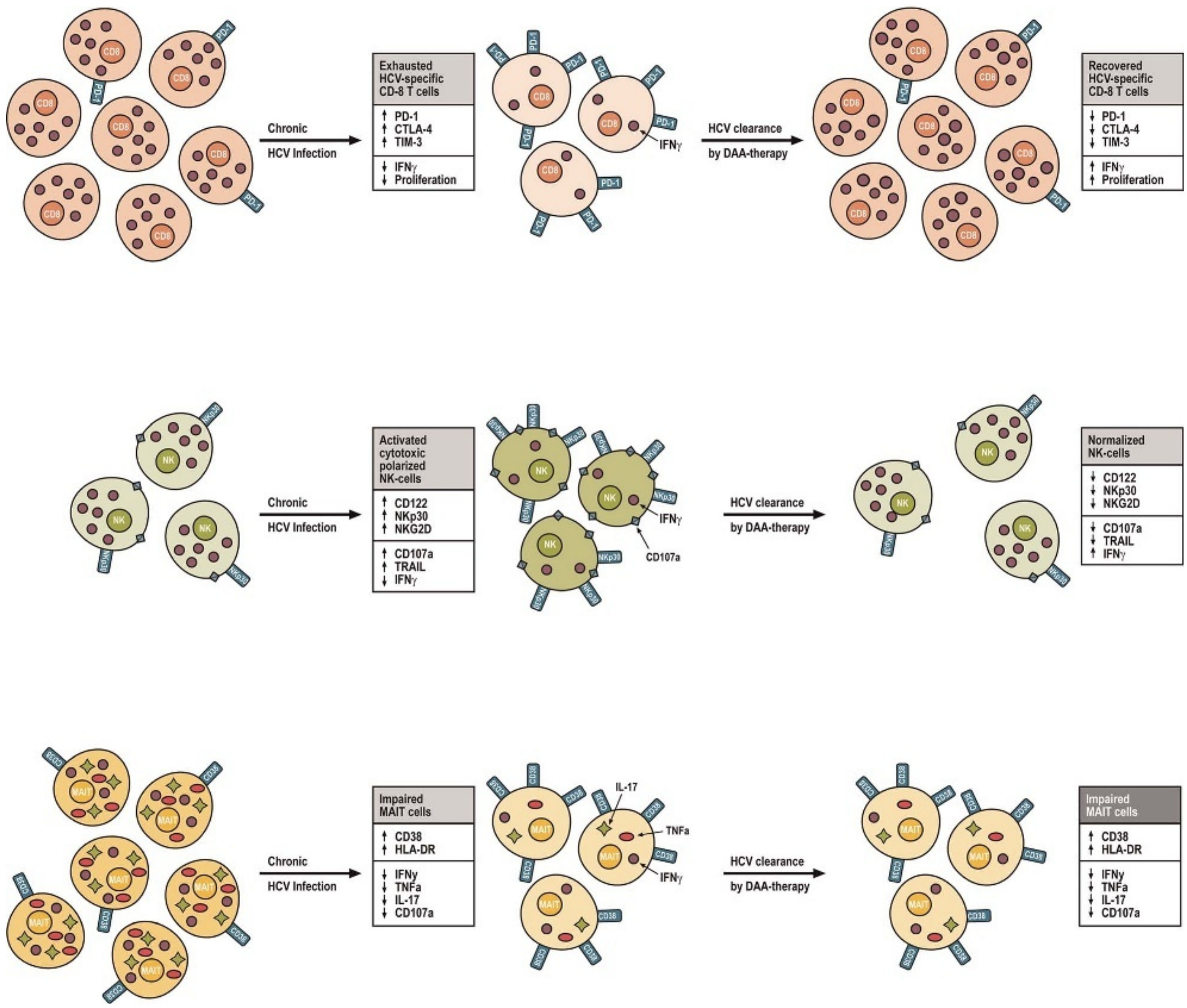

FIGURE 1. Change of immune cell function after DAA-mediated HCV clearance.

Furthermore, responsiveness of NK cells to endogenous IFN is improved when IFN homeostasis is restored in the liver after disrupting HCV-induced activation of the innate immune response.[39]

In sharp contrast to the observed recovery of virus-specific CD8+ T cells and NK cells, DAA therapy in HCV patients does not lead to a regeneration of the MAIT cell compartment. Peripheral MAIT cells remained at a low frequency in a study where all 8 successfully treated chronically HCV-infected patients were followed up long term and did not reach levels observed in healthy donors. Furthermore, MAIT cells did not show a significant recovery in MR1-dependent cytokine production or cytotoxicity after DAA-mediated HCV clearance.[20] In contrast, IFN-based therapies have been recently shown to increase MAIT-cell activation and frequency in HCV patients after successful treatment.[19]

THE ROLE OF IMMUNE RESPONSES IN HEPATOCARCINOGENESIS

The liver immune network plays a central role in the pathogenesis of HCC, both positive and negative, depending on the prevailing conditions in the patient. On the one hand it is supporting tumor growth and angiogenesis with tumor-infiltrating macrophages and neutrophils; on the other hand, it also exhibits a protective role in tumor surveillance via antitumor CD8+ T cells and NK cells.[40]

To mediate an antitumor effect, immune cells need to penetrate the tumor tissue. Indeed, infiltration of HCC lesions by lymphocytes correlates with a reduced risk of HCC recurrence.[41] CD8+ T cells specific for tumor-associated antigens thereby are the main antitumor effector cells.[42] Interestingly, the breadth of tumor-associated antigen-specific immune responses is an independent prognostic factor for patient survival.[43] Furthermore, an increase in the frequency of circulating CD4 + CD25 + FoxP3+ Treg cells has been observed as HCC progressed, which negatively affects the functions of the T cells and is inversely correlated with the number of the tumor-specific CD8+ T cells.[44] This increase in the frequency of Treg correlates with the frequency of myeloid-derived suppressor cell defined as CD14-HLADR-CD11b + CD33+ cells, and expression of CTLA-4 and PD-1 on CD4+ T cells.[45,46] However, although tumor-associated antigen-specific CD8+ T cells have not yet been studied during DAA-therapy of HCV, it has been shown that the CD45RA-CCR7- effector memory compartment of CD4+ and CD8+ T cells increases and expression of the T cell coinhibitory receptor TIGIT (T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif [ITIM] domain) decreases on the bulk CD3+ T cell population.[34] TIGIT negatively regulates antitumor responses, whereas it is highly expressed on tumor infiltrating lymphocytes.[47]

NK cells not only represent the first line of defense against intruding viruses, they also have an anti-tumor effect by direct lysis of malignant cells.[48] Consequently, frequency of NK cells in the peripheral blood of patients with HCC positively correlates with recurrence-free survival; specifically, $CD56^{dim}$ NK cells have been found to be decreased in HCC patients.[49,50] Moreover, NK cells often exhibit impaired functional activities in advanced-stage HCC patients and cytotoxicity of NK cells from both the peripheral blood and tumor sites of HCC patients is notably reduced compared with healthy controls.[50,51] Because NK cells mediate target-cell apoptosis through surface expression of TRAIL,[52] it is interesting that peripheral as well as intrahepatic NK cells of HCV patients show a decrease in TRAIL expression during DAA therapy.[37,38] Furthermore, IFN-γ production of $CD56^{bright}$ NK cells increased in these patients while cytotoxicity of $CD56^{dim}$ NK cells measured (indicated by the degranulation marker CD107a) decreased after DAA-mediated clearance of HCV.[37]

In contrast to the other immune cell populations studied, MAIT cells do not increase in frequency or function after successful DAA therapy.[20] In this context, it is notable that circulating MAIT cells have recently been reported to be diminished in mucosal-associated cancer, but may participate in tumor immunosurveillance by infiltration and IL-17-mediated cell cycle arrest.[53] Their role in HCC development or control, however, is unknown.

Taken together, in chronic HCV infection constant antigen stimulation contributes to virus-specific CD8+ T cell exhaustion and continues to activate host-mediated inflammation in the liver, in part driven by endogenous IFNs, and alters innate immune cell populations such as NK cells and MAIT cells. IFN-free DAA-therapy regimens for HCV infection provide a unique opportunity to study the interaction between HCV and the intrahepatic immune system because these regimens rapidly decrease viremia to undetectable levels. In this context, it has been shown that DAA-mediated HCV clearance restores frequency and function of certain immune cells, such as virus-specific CD8+ T cells and NK cells. Furthermore, the lack of continuous IFN stimulation in the liver after elimination of the virus will likely also have a significant impact on intrahepatic immune responses. Because balancing immune responses is a dynamic process of immune activation and immune regulation, the immune response is in a precarious state for a certain time span. Thus, without further studies, it remains speculative whether the rapid decrease in NK cell activation and cytotoxicity after DAA therapy, nonreversible MAIT cell dysfunction or the "normalized" liver microenvironment may support HCC progression by disrupting immunological balances in the liver.

REFERENCES

1. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *J Hepatol*. 2014;61(1 Suppl):S58–S68.
2. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med*. 2011;365:1118–1127.
3. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65:87–108.
4. Curry MP, Forns X, Chung RT, et al. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology*. 2015;148:100–107 e101.
5. Conti F, Buonfiglioli F, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol*. 2016;65:727–733.
6. Reig M, Mariño Z, Perelló C, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol*. 2016;65:719–726.
7. ANRS collaborative study group. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. *J Hepatol*. 2016;65:734–740.
8. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA*. 2012;308:2584–2593.
9. Hsu CS, Chao YC, Lin HH, et al. Systematic review: impact of interferon-based therapy on HCV-related hepatocellular carcinoma. *Sci Rep*. 2015;5:9954.
10. Meissner EG, Wu D, Osinusi A, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. *J Clin Invest*. 2014;124:3352–3363.
11. Wieland S, Makowska Z, Campana B, et al. Simultaneous detection of hepatitis C virus and interferon stimulated gene expression in infected human liver. *Hepatology*. 2014;59:2121–2130.
12. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci U S A*. 2008;105:7034–7039.
13. Bohne F, Londoño MC, Benítez C, et al. HCV-induced immune responses influence the development of operational tolerance after liver transplantation in humans. *Sci Transl Med*. 2014;6:1–13.
14. Rehermann B. Natural killer cells in viral hepatitis. *Cell Mol Gastroenterol Hepatol*. 2015;1:578–588.
15. Cashman SB, Marsden BD, Dustin LB. The humoral immune response to HCV: understanding is key to vaccine development. *Front Immunol*. 2014;5:550.
16. Rehermann B. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. *Nat Med*. 2013;19:859–868.
17. Godfrey DI, Uldrich AP, McCluskey J, et al. The burgeoning family of unconventional T cells. *Nat Immunol*. 2015;16:1114–1123.
18. Barathan M, Mohamed R, Vadivelu J, et al. Peripheral loss of CD8(+) CD161(++) TCRVα7·2(+) mucosal-associated invariant T cells in chronic hepatitis C virus-infected patients. *Eur J Clin Invest*. 2016;46:170–180.
19. van Wilgenburg B, Scherwitzl I, Hutchinson EC, et al. MAIT cells are activated during human viral infections. *Nat Commun*. 2016;7:11653.
20. Hengst J, Strunz B, Deterding K, et al. Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. *Eur J Immunol*. 2016;46:2204–2210.
21. Saha B, Szabo G. Innate immune cell networking in hepatitis C virus infection. *J Leukoc Biol*. 2014;96:757–766.
22. Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. *J Hepatol*. 2014;60:1090–1096.
23. Boltjes A, Movita D, Boonstra A, et al. The role of Kupffer cells in hepatitis B and hepatitis C virus infections. *J Hepatol*. 2014;61:660–671.
24. Khakoo SI, Soni PN, Savage K, et al. Lymphocyte and macrophage phenotypes in chronic hepatitis C infection. Correlation with disease activity. *Am J Pathol*. 1997;150:963–970.
25. Dolganiuc A, Norkina O, Kodys K, et al. Viral and host factors induce macrophage activation and loss of toll-like receptor tolerance in chronic HCV infection. *Gastroenterology*. 2007;133:1627–1636.
26. Shrivastava S, Mukherjee A, Ray R, et al. Hepatitis C virus induces interleukin-1β (IL-1β)/IL-18 in circulatory and resident liver macrophages. *J Virol*. 2013;87:12284–12290.
27. Negash AA, Ramos HJ, Crochet N, et al. IL-1β production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. *PLoS Pathog*. 2013;9:e1003330.
28. Serti E, Werner JM, Chattergoon M, et al. Monocytes activate natural killer cells via inflammasome-induced interleukin 18 in response to hepatitis C virus replication. *Gastroenterology*. 2014;147:209–220 e203.

29. Zoulim F, Liang TJ, Gerbes AL, et al. Hepatitis C virus treatment in the real world: optimising treatment and access to therapies. *Gut*. 2015;64: 1824–1833.
30. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. *N Engl J Med*. 2013;368:1907–1917.
31. Aa IDSA. American Association for the Study of Liver Diseases and Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C. http://www.hcvguidelines.org/full-report-view. Published 2016. Accessed 06/20/16.
32. Werner JM, Vermehren J. New HCV therapies and liver transplantation. *Transplantation*. 2016;100:260–261.
33. Martin B, Hennecke N, Lohmann V, et al. Restoration of HCV-specific CD8 + T cell function by interferon-free therapy. *J Hepatol*. 2014;61:538–543.
34. Burchill MA, Golden-Mason L, Wind-Rotolo M, et al. Memory re-differentiation and reduced lymphocyte activation in chronic HCV-infected patients receiving direct-acting antivirals. *J Viral Hepat*. 2015;22:983–991.
35. Callendret B, Eccleston HB, Hall S, et al. T-cell immunity and hepatitis C virus reinfection after cure of chronic hepatitis C with an interferon-free antiviral regimen in a chimpanzee. *Hepatology*. 2014;60:1531–1540.
36. Carlin AF, Aristizabal P, Song Q, et al. Temporal dynamics of inflammatory cytokines/chemokines during sofosbuvir and ribavirin therapy for genotype 2 and 3 hepatitis C infection. *Hepatology*. 2015;62:1047–1058.
37. Serti E, Chepa-Lotrea X, Kim YJ, et al. Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function. *Gastroenterology*. 2015;149:190–200 e192.
38. Spaan M, van Oord G, Kreefft K, 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.
39. Serti E, Park H, Keane M, et al. Rapid decrease in hepatitis C viremia by direct acting antivirals improves the natural killer cell response to IFNα. *Gut*. 2016.
40. Schmidt N, Thimme R. Role of immunity in pathogenesis and treatment of hepatocellular carcinoma. *Dig Dis*. 2016;34:429–437.
41. Yoong KF, McNab G, Hübscher SG, et al. Vascular adhesion protein-1 and ICAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma. *J Immunol*. 1998;160:3978–3988.
42. Wada Y, Nakashima O, Kutami R, et al. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology*. 1998;27: 407–414.
43. Flecken T, Schmidt N, Hild S, et al. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. *Hepatology*. 2014;59:1415–1426.
44. Fu J, Xu D, Liu Z, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology*. 2007;132:2328–2339.
45. Lugade AA, Kalathil S, Miller A, et al. High immunosuppressive burden in advanced hepatocellular carcinoma patients: can effector functions be restored? *Oncoimmunology*. 2013;2:e24679.
46. Kalathil SG, Lugade AA, Miller A, et al. PD-1+ and Foxp3+ T cell reduction correlates with survival of HCC patients after sorafenib therapy. *JCI Insight*. 2016;1.
47. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity*. 2016;44:989–1004.
48. Pahl J, Cerwenka A. Tricking the balance: NK cells in anti-cancer immunity. *Immunobiology*. 2017;222:11–20 pii:S0171-2985(15):30031–30030.
49. Taketomi A, Shimada M, Shirabe K, et al. Natural killer cell activity in patients with hepatocellular carcinoma: a new prognostic indicator after hepatectomy. *Cancer*. 1998;83:58–63.
50. Cai L, Zhang Z, Zhou L, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol*. 2008;129:428–437.
51. Hoechst B, Voigtlaender T, Ormandy L, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology*. 2009;50:799–807.
52. Smyth MJ, Cretney E, Kelly JM, et al. Activation of NK cell cytotoxicity. *Mol Immunol*. 2005;42:501–510.
53. Ling L, Lin Y, Zheng W, et al. Circulating and tumor-infiltrating mucosal associated invariant T (MAIT) cells in colorectal cancer patients. *Sci Rep*. 2016;6:20358.