New Therapies, Markers and Therapeutic Targets in HCV Chronic Infection, and HCV Extrahepatic Manifestations.

Alessandro Antonelli [1], Mauro Pistello [2]

[1] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy;

[2] Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy.

Email Addresses:
Alessandro Antonelli: alessandro.antonelli@med.unipi.it
Mauro Pistello: mauro.pistello@med.unipi.it

Address correspondence to:
Alessandro Antonelli, Prof
Department of Clinical and Experimental Medicine,
University of Pisa,
Via Savi, 10, I-56126, Pisa, Italy
Tel: +39-050-992318; Fax: +39-050-553235;
e-mail: alessandro.antonelli@med.unipi.it

Abstract

More than 180 millions of subjects in the world are infected by Hepatitis C Virus (HCV), and about 20% of them with HCV chronic infection progress to cirrhosis. Furthermore, numerous HCV extrahepatic manifestations have been reported in up to 74% of patients, as mixed cryoglobulinemia, lymphomas, rheumatic disorders, autoimmune thyroiditis, hypothyroidism, papillary thyroid cancer, and type 2 diabetes. Advances in understanding the HCV life cycle, and the inflammatory processes (involving a complex network of cytokines and chemokines) associated with HCV chronic infection, have led to substantial advancements in therapy. **The combination of ribavirin and PEGylated interferon-α was the standard of therapy for HCV chronically infected patients in the last decades. However,** interferon has limited effectiveness and is associated with severe adverse effects. **Recently, direct-acting antivirals (DAAs) that act as inhibitors of N5SA, or polymerase, or protease have been shown to result in shorter duration of therapy, better efficacy and tolerance, with respect to ribavirin and PEGylated interferon-α.** Circulating CXCL10 levels, and **the interleukin(IL)-28B gene polymorphisms,** are associated with the **success of the therapy both with DAAs or ribavirin and PEGylated interferon-alpha. New DAAs targeting the HCV at various molecular levels have been developed to eradicate HCV.** Moving to **interferon-**free therapies should offer new treatments for resistant HCV genotypes, and for ineligible patients or patients failing to respond to prior therapies.

Many efforts have been made to understand the factors that are involved with clearance of HCV to personalize the therapy for each patient, with the aim to reduce side effects, increasing the sustained virologic response rate, and to prevent the progression of the disease.

EDITORIAL

More than 180 millions of subjects in the world are infected by Hepatitis C Virus (HCV), and about 20% of them with HCV chronic infection progress to cirrhosis [1]. Furthermore, numerous extrahepatic manifestations (HCV-EHMs) have been reported in up to 74% of patients [2-5]. HCV strategies that limit or delay the initiation of innate antiviral responses are important in determining the final outcome of the infection [6]. High rate of replication and error prone genome replication machinery enable HCV to evade immune recognition. This delay of an effective immune response allows HCV to establish widespread infection in liver and extra-hepatic tissues and cells. Changing viral epitopes induces a failure of the adaptive immune response. Moreover, neutralizing antibody epitopes may be hidden by decoy structures, lipoproteins and glycans [7, 8]. Also, T cell responses fail due to changing viral epitope sequences; while the phenomenon of exhaustion is probably evolved to limit immune-mediated pathology [9]. As a result, immune-mediated clearance of HCV infection is occurring only in about 20% of people, by innate and adaptive immune mechanisms [10].

A complex orchestration of cytokines and chemokines coordinate immune response, both innate and adaptive, in the initial phases of HCV infection, and plays a pivotal role in controlling viral replication and liver damage [11]. Among cytokines, it is well known that the production of and/or response to interferons (IFNs) are very important in the induction of the immune response during chronic HCV infection (CHC) [12, 13], and several studies have shown that patients with CHC have a deregulated IFNs response [14]. In fact, patients who already have a high level of endogenous IFN-stimulated genes (ISGs) expression do not achieve viral clearance or have a poor response to treatment with IFN-α [15]. The viral and/or host factors that are responsible for the greater baseline ISGs expression in certain HCV patients remain to be determined [16]. Recently, it has been shown that type III IFNs, and in particular the new discovered IFN-λ4, play a dominant role in driving ISGs response and in contributing to the viral clearance or persistence [17-19].

A predominance of the Th1 immune response (and related cytokines/chemokines) has been shown in CHC and in HCV-EHMs [20, 21]. **Interferons, and chemokines inducible by interferon-γ [(C-X-C motif) ligand (CXCL)9, -10 and -11] recruit inflammatory cells into the hepatic parenchyma (when the infection is not controlled) producing chronic inflammation and fibrosis of the liver, that may results in hepatic cirrhosis** [22-25]**. A complex dysregulation of cytokines and chemokines response is associated with HCV systemic manifestations [mixed cryoglobulinemia (MC), lymphomas, autoimmune diseases of the thyroid (AITD), type 2 diabetes], and involves overall the Th1 cyokines/chemokines** [23, 26]. **HCV escapes immunity interfering at various levels with cytokines/chemokines, and inducing a Th2/Tc2 immune response** [26, 27]. **The administration of interferon-α can induce the HCV clearance during CHC, and can revert the progression to cirrhosis, reducing CXCL10 levels [**25, 28]. **It has been suggested that agents directed to neutralize CXCL10 could increase the responsiveness of patients to traditional therapies of HCV infection,** simultaneously reducing inflammatory immune cell activation [29]. **Many studies have shown that both circulating CXCL10, and polymorphisms of interleukin (IL)-28B, could be used as prognostic markers of efficacy of HCV therapies [30-33].** Other studies have also shown that HCV clearance by direct-acting antiviral (DAAs) therapies decreases circulating CXCL10 levels [34].

HCV infection regulates a number of microRNAs (miRNAs), which are able to exert an effect on liver biology and pathology [35, 36]. In HCV infected hepatocytes miRNAs can directly regulate HCV replication through interaction with the HCV genome [37]. Moreover miRNAs induced by HCV can indirectly control critical virus-associated host pathways, inducing liver fibrosis, cirrhosis, and/or hepatocellular carcinoma [38, 39]. Recently, circulating miRNAs are emerging as biomarkers for HCV associated disorders. Considerable efforts have been employed to investigate the change in the circulating miRNA pattern **in HCV infection and related diseases** [40, 41]. **Distinctive circulating miRNA patterns are associated with**

HCV infection and HCV-related hepatic diseases [42], suggesting that miRNAs are non-invasive biomarkers to evaluate the diagnosis and prognosis of these disorders [43].

Treatment for HCV infection has recently progressed from poorly tolerated IFN-α therapy and with very low cure rates, to highly effective oral DAAs with cure rates above 90% for almost all patients, and with little adverse effects [44-46]. Understanding of the viral lifecycle, with recognition of targets that could be inhibited by small molecules, **has permitted the production of DAAs, that inhibit protease, non-structural 5a (NS5A), and nucleotide and non-nucleotide polymerase [47-49].** Initially DAAs have been used **with Pegylated-Interferon-α (Peg- IFN) and Ribavirin (RBV),** and subsequently **in** combination without the need for IFNs [50]. Rational DAAs combinations have overcome the major challenge of rapid emergence of drug resistance [51]. Second-generation DAAs agents in each class have further improved safety and efficacy profiles, reducing drug-drug interactions and adverse side effects [52-54].

In the new era of DAAs therapy in which elimination of HCV infection is a real possibility, HCV infected cell culture models are important for the identification of therapeutic targets, testing candidate drugs, and profiling of therapeutic strategies [55-58]. The development of protocols to grow HCV in culture and generate hepatocyte cell lines from specific individuals holds great promise to investigate the mechanisms exploited by the virus to spread the infection and the host factors critical for HCV replication and propagation, or resistance to infection [59-63]. These models could be used for the development of drugs targeting host factors essential for virus replication, holding great promises in further increasing treatment efficacy [64].

The most common HCV-EHMs of HCV infection is MC syndrome (MCs), an immune-comp**lex mediated vasculitis, involving joints, skin, peripheral nerves, and internal organs.** In **the** majority of individuals, MCs shows a mild, slow-progressive clinical course needing only symptomatic treatments [65-68]. However the etiologic therapy was considered the first-line option in MCs patients and, in the past two decades, antiviral treatment with **RBV plus Peg-IFN represented the standard of care** [69]. Rapidly progressive, diffuse MC

vasculitis with multiple organ involvement may be successfully treated with aggressive immunosuppressive and anti-inflammatory therapies, mainly based on cyclophosphamide or rituximab, high dose corticosteroids, and plasmapheresis [70]. The recent introduction of DAAs substantially changed the treatment of HCV infection. DAAs anti-HCV therapy seem to be safe and effective in patients with MC and MCs from the virological and clinical points of view, thus confirming the key role played by HCV eradication in inducing MC remission [71-73]. **The combination, or a sequence, of antiviral and immunosuppressive treatments has been shown to be useful in the therapy of MCs patients with major clinical manifestations. In the clinical practice, treatment of MCs needs to be personalized for each patient in relation to clinical symptoms and the severity of the diseases** [74-76].

On the bases of epidemiological data, biological investigation, as well as clinical observations, HCV is increasingly recognized to be involved in the pathogenesis of a variety of histological **types of B-cell non-Hodgkin'** s **lymphoma (B-NHL)** [77-80]. **To understand the mechanisms related to HCV persistence, and lymphomagenesis, is necessary to develop new therapies with the aim to prevent and to treat B-NHLs [81, 82].** It has been also observed that at least some types of HCV-related indolent B-NHLs disappear after successful antiviral treatment, strongly reinforcing the hypothesis of a HCV-induced lymphomagenesis [83]. The role of DAAs in the prevention and treatment of HCV associated lymphoproliferation remains to be clarified [84, 85].

Other HCV-EHMs are various rheumatic disorders (mainly arthritis, sicca syndrome, osteosclerosis, etc) [86-91]. The management of HCV-associated rheumatic diseases is particularly difficult **because of the coexistence of complex immunological disorders and HCV infection, and needs to be personalized in each patient [92-94].**

The occurrence of autoimmune thyroiditis, hypothyroidism, papillary thyroid cancer and type 2 diabetes is also more frequent in CHC: these disorders can significantly impact the quality of

life of HCV patients, the course of the disease, and the effect of therapies [95-98]. The role of DAAs in the prevention and treatment of these endocrine disorders remains to be clarified.

In this issue, we focus on the recent advancements in the viral and host factors influencing their interplay, highlighting current knowledge in the inflammatory processes (and the inherent network of cytokines and chemokines) associated with CHC, and illustrate the multifaceted HCV-EHMs, that heavily affect the quality of life of HCV patients. We then describe how the development of reliable systems to propagate the virus in vitro and the identification of small molecules targeting key steps of HCV replication have led to substantial advancements in the therapy. Finally, we give an overview of current approaches for treatment and of novel DAAs targeting **various stages of the life of HCV** and holding promise **to eradicate HCV infection.** Moving to IFN-free therapies should offer new treatments for resistant HCV genotypes, and for ineligible patients or patients failing to respond to prior therapies. **The knowledge of viral and host factors associated with the clearance of HCV is very important to personalize the therapy in each patient, increasing the sustained virologic response rates, preventing the progression of hepatic disease, and reducing adverse side effects.**

Conflict of Interest

The authors have no conflict of interests to declare.

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References:

[1] Sarna A, Panda S. HCV in people who inject drugs: a neglected epidemic. Lancet Infect Dis 2015; 15: 4-5.

[2] Rosenthal E, Cacoub P. Extrahepatic manifestations in chronic hepatitis C virus carriers. Lupus 2015; 24: 469-82.

[3] Ferrari SM, Fallahi P, Mancusi C, *et al.* HCV-related autoimmune disorders in HCV chronic infection. Clin Ter 2013; 164: e305-12.

[4] Matthews PC, Jeffery K, Klenerman P, Barnes E, Cooke G. Screening and treatment for hepatitis C: a balanced perspective. BMJ 2015; 350: h644.

[5] Ward CJ, Lee V. Hepatitis C screening of men who have sex with men. BMJ 2015; 350: h642.

[6] Yang DR, Zhu HZ. Hepatitis C virus and antiviral innate immunity: who wins at tug-of-war? World J Gastroenterol 2015; 21: 3786-800.

[7] Vermehren J, Sarrazin C. The role of resistance in HCV treatment. Best Pract Res Clin Gastroenterol 2012; 26: 487-503.

[8] Dustin LB. Innate and Adaptive Immune Responses in Chronic HCV infection. Curr Drug Targets, in press.

[9] Dustin LB, Cashman SB, Laidlaw SM. Immune control and failure in HCV infection--tipping the balance. J Leukoc Biol 2014; 96: 535-48.

[10]Larrubia JR, Moreno-Cubero E, Lokhande MU, *et al.* Adaptive immune response during hepatitis C virus infection. World J Gastroenterol 2014; 20: 3418-30.

[11]Fallahi P, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. Clin Dev Immunol 2012; 2012: 468107.

[12]Scagnolari C, Antonelli G. Antiviral activity of the interferon α family: biological and pharmacological aspects of the treatment of chronic hepatitis C. Expert Opin Biol Ther 2013; 13: 693-711.

[13]Antonelli G. Biological basis for a proper clinical application of alpha interferons. New Microbiol 2008; 31: 305-18.

[14]Zhou Y, Zhang Y, Moorman JP, Yao ZQ, Jia ZS. Viral (hepatitis C virus, hepatitis B virus, HIV) persistence and immune homeostasis. Immunology 2014; 143: 319-30

[15]Scagnolari C, Monteleone K, Cacciotti G, Antonelli G. Role of interferons in chronic hepatitis C infection. Curr Drug Targets, in press.

[16]Metz P, Reuter A, Bender S, Bartenschlager R. Interferon-stimulated genes and their role in controlling hepatitis C virus. J Hepatol 2013; 59: 1331-41.

[17]Laidlaw SM, Dustin LB. Interferon lambda: opportunities, risks, and uncertainties in the fight against HCV. Front Immunol 2014; 5: 545.

[18]Lin FC, Young HA. Interferons: Success in anti-viral immunotherapy. Cytokine Growth Factor Rev 2014; 25: 369-76.

[19]Egli A, Santer DM, O'Shea D, Tyrrell DL, Houghton M. The impact of the interferon-lambda family on the innate and adaptive immune response to viral infections. Emerg Microbes Infect 2014; 3: e51.

[20]Antonelli A, Ferrari SM, Ruffilli I, Fallahi P. Cytokines and HCV-related autoimmune disorders. Immunol Res 2014; 60: 311-9.

[21]Brownell J, Polyak SJ. Molecular pathways: hepatitis C virus, CXCL10, and the inflammatory road to liver cancer. Clin Cancer Res 2013; 19: 1347-52.

[22]Marra F, Tacke F. Roles for chemokines in liver disease. Gastroenterology 2014; 147: 577-594.

[23]Brass A, Brenndörfer ED. The role of chemokines in hepatitis C virus-mediated liver disease. Int J Mol Sci 2014; 15: 4747-79.

[24]Antonelli A, Fallahi P, Ferrari SM, *et al.* Serum Th1 (CXCL10) and Th2 (CCL2) chemokine levels in children with newly diagnosed Type 1 diabetes: a longitudinal study. Diabet Med 2008; 25: 1349-53.

[25]Fahey S, Dempsey E, Long A. The role of chemokines in acute and chronic hepatitis C infection. Cell Mol Immunol 2014; 11: 25-40.

[26]Antonelli A, Ferri C, Fallahi P, *et al.* Alpha-chemokine CXCL10 and beta-chemokine CCL2 serum levels in patients with hepatitis C-associated cryoglobulinemia in the presence or absence of autoimmune thyroiditis. Metabolism 2008; 57: 1270-7.

[27]Oo YH, Shetty S, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. Dig Dis 2010; 28: 31-44.

[28]Parekh PJ, Shiffman ML. The role of interferon in the new era of hepatitis C treatments. Expert Rev Gastroenterol Hepatol 2014; 8: 649-56.

[29] Fallahi P, Ferrari SM, Giuggioli D, *et al.* Chemokines in the pathogenesis and as therapeutical markers and targets of HCV chronic infection, and HCV extrahepatic manifestations. Curr Drug Targets, in press.

[30]Lee DH, Lee JH, Kim YJ, *et al.* Relationship between polymorphisms near the IL28B gene and spontaneous HBsAg seroclearance: a systematic review and meta-analysis. J Viral Hepat 2014; 21: 163-70.

[31]Mangia A, Mottola L, Santoro R. Interleukin 28B polymorphisms as predictor of response in hepatitis C virus genotype 2 and 3 infected patients. World J Gastroenterol 2013; 19: 8924-8.

[32]Bibert S, Roger T, Calandra T, et al. IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. J Exp Med 2013; 210: 1109-16.

[33]Butera D, Marukian S, Iwamaye AE, et al. Plasma chemokine levels correlate with the outcome of antiviral therapy in patients with hepatitis C. Blood. 2005; 106:1175-82.

[34]Schaefer CJ, Kossen K, Lim SR, et al. Danoprevir monotherapy decreases inflammatory markers in patients with chronic hepatitis C virus infection. Antimicrob Agents Chemother 2011; 55: 3125-32.

[35]Waldron PR, Holodniy M. MicroRNA and hepatitis C virus--challenges in investigation and translation: a review of the literature. Diagn Microbiol Infect Dis 2014; 80: 1-12.

[36]Lee CH, Kim JH, Lee SW. The role of microRNA in pathogenesis and as markers of HCV chronic infection. Curr Drug Targets, in press.

[37]Lee CH, Kim JH, Lee SW. The role of microRNAs in hepatitis C virus replication and related liver diseases. J Microbiol 2014; 52: 445-51.

[38]Xin X, Zhang Y, Liu X, Xin H, Cao Y, Geng M. MicroRNA in hepatic fibrosis and cirrhosis. Front Biosci (Landmark Ed) 2014; 19: 1418-24.

[39]Greene CM, Varley RB, Lawless MW. MicroRNAs and liver cancer associated with iron overload: therapeutic targets unravelled. World J Gastroenterol 2013; 19: 5212-26.

[40]Zhang Y, Wang Z, Gemeinhart RA. Progress in microRNA delivery. J Control Release 2013; 172: 962-74.

[41]Fukuhara T, Kambara H, Shiokawa M, et al. Expression of microRNA miR-122 facilitates an efficient replication in nonhepatic cells upon infection with hepatitis C virus. J Virol 2012; 86: 7918-33.

[42]Fognani E, Giannini C, Piluso A, et al. Role of microRNA profile modifications in hepatitis C virus-related mixed cryoglobulinemia. PLoS One 2013; 8: e62965.

[43]Augello C, Gianelli U, Savi F, et al. MicroRNA as potential biomarker in HCV-associated diffuse large B-cell lymphoma. J Clin Pathol 2014; 67: 697-701.

[44]Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. Lancet 2015; 385: 1124-35.

[45]Verstegen MM, Pan Q, van der Laan LJ. Gene therapies for hepatitis C virus. Adv Exp Med Biol 2015; 848: 1-29.

[46]Feld JJ. Interferon-free strategies with a nucleoside/nucleotide analogue. Semin Liver Dis 2014; 34: 37-46.

[47]Janardhan SV, Reau NS. Should NS5A inhibitors serve as the scaffold for all-oral anti-HCV combination therapies? Hepat Med 2015; 7: 11-20.

[48]Nakamoto S, Kanda T, Shirasawa H, Yokosuka O. Antiviral therapies for chronic hepatitis C virus infection with cirrhosis. World J Hepatol 2015; 7: 1133-41.

[49]Feeney ER, Chung RT. Antiviral treatment of hepatitis C. BMJ 2014; 348: g3308.

[50]Aronsohn A, Jensen D. Interferon-combination strategies for the treatment of chronic hepatitis C. Semin Liver Dis 2014; 34: 30-6.

[51]Kumari R, Nguyen MH. Fixed-dose combination of sofosbuvir and ledipasvir for the treatment of chronic hepatitis C genotype 1. Expert Opin Pharmacother 2015; 16: 739-48.

[52]Campagna D, Demma S. Faldaprevir for the treatment of hepatitis C. Drugs Today (Barc) 2015; 51: 289-301.

[53]Kim do Y, Ahn SH, Han KH. Emerging therapies for hepatitis C. Gut Liver 2014; 8: 471-9.

[54]Feld J. Direct-acting antivirals for hepatitis C virus (HCV): The progress continues. Curr Drug Targets, in press.

[55]Park EM, Lim YS, Ahn BY, Hwang SB. AAM-B Interacts with Nonstructural 4B and Regulates Hepatitis C Virus Propagation. PLoS One 2015; 10: e0132839.

[56]Morrica A, Giorgi M, Maggi F, et al. Susceptibility of human and non-human cell lines to HCV infection as determined by the centrifugation-facilitated method. J Virol Methods 1999; 77: 207-15.

[57]Maggi F, Fornai C, Morrica A, et al. Divergent evolution of hepatitis C virus in liver and peripheral blood mononuclear cells of infected patients. J Med Virol 1999; 57: 57-63.

[58]Chen S, Li S, Chen L. Interferon-inducible protein 6-16 (IFI-6-16, ISG16) promotes hepatitis C virus replication in vitro. J Med Virol 2015, Epub ahead of print.

[59]Tawar RG, Colpitts CC, Timm J, et al. Acute hepatitis C virus infection induces anti-host cell receptor antibodies with virus-neutralizing properties. Hepatology 2015, Epub ahead of print.

[60]Kurt R, Chandra PK, Aboulnasr F, et al. Chaperone-Mediated Autophagy Targets IFNAR1 for Lysosomal Degradation in Free Fatty Acid Treated HCV Cell Culture. PLoS One 2015; 10: e0125962.

[61]Von Schaewen M, Ding Q, Ploss A. Visualizing hepatitis C virus infection in humanized mice. J Immunol Methods 2014; 410: 50-9.

[62]Taylor DR. Evolution of cell culture systems for HCV. Antivir Ther 2013; 18: 523-30.

[63]Catanese MT, Dorner M. Advances in experimental systems to study hepatitis C virus in vitro and in vivo. Virology 2015; 479-480: 221-33.

[64]Maggi F, Focosi D, Pistello M. How Current Direct-Acting Antiviral and Novel Cell Culture Systems for HCV are Shaping Therapy and Molecular Diagnosis of Chronic HCV Infection. Curr Drug Targets, in press.

[65]Ferri C, Antonelli A, Mascia MT, *et al*. B-cells and mixed cryoglobulinemia. Autoimmun Rev 2007; 7:114-20.

[66]Pietrogrande M, De Vita S, Zignego AL, *et al*. Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients. Autoimmun Rev 2011;10: 444-54.

[67]Ferri C. Mixed cryoglobulinemia. Orphanet J Rare Dis 2008;3 :25.

[68]Cacoub P, Comarmond C, Domont F, Savey L, Saadoun D. Cryoglobulinemia Vasculitis. Am J Med 2015.

[69]Saadoun D, Delluc A, Piette JC, Cacoub P. Treatment of hepatitis C-associated mixed cryoglobulinemia vasculitis. Curr Opin Rheumatol 2008; 20: 23-8.

[70]Giuggioli D, Manfredi A, Colaci M, *et al*. Systemic sclerosis and cryoglobulinemia: our experience with overlapping syndrome of scleroderma and severe cryoglobulinemic vasculitis and review of the literature. Autoimmun Rev 2013; 12: 1058-63.

[71]Gragnani L, Fabbrizzi A, Triboli E *et al*.Triple antiviral therapy in hepatitis C virus infection with or without mixed cryoglobulinaemia: a prospective, controlled pilot study. Dig Liver Dis 2014; 46 :833-7.

[72]Cornella SL, Stine JG, Kelly V, Caldwell SH, Shah NL. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: A case series. Postgrad Med 2015; 127 :413-7.

[73]Urraro T, Gragnani L, Piluso A, *et al*. Combined treatment with antiviral therapy and rituximab in patients with mixed cryoglobulinemia: review of the literature and report of a case using direct antiviral agents-based antihepatitis C virus therapy. Case Reports Immunol 2015; 2015 :816424.

[74]Gragnani L, Piluso A, Urraro T, *et al*. Virological and Clinical Response in Patients with HCV-Related Mixed Cryoglobulinemia Treated with Interferon-Free Regimens: Preliminary Results of a Prospective Pilot Study. Curr Drug Targets, in press**.**

[75]Dammacco F, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. N Engl J Med 2013; 369 :1035-45.

[76]Humphries K, Darling JM, Barritt AS. Membranoproliferative glomerulonephritis, type II cryoglobulinemia and triple therapy for hepatitis C: a case series and review of the literature. Dig Dis Sci 2014; 59 :2007-12.

[77]Ferri C, Monti M, La Civita L, *et al*. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. Blood 1993; 82: 3701-4.

[78]Paydas S. Hepatitis C virus and lymphoma. Crit Rev Oncol Hematol 2015; 93: 246-56.

[79]Ferri C, Antonelli A, Mascia MT, *et al*. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. Dig Liver Dis 2007; 1: 13-21.

[80]Kondo Y, Shimosegawa T. Direct effects of hepatitis C virus on the lymphoid cells. World J Gastroenterol 2013; 19: 7889-95.

[81]Vannata B, Stathis A, Zucca E. Management of the marginal zone lymphomas. Cancer Treat Res 2015; 165: 227-49.

[82]Negro F. Hepatitis C in 2013: HCV causes systemic disorders that can be cured. Nat Rev Gastroenterol Hepatol 2014; 11: 77-8.

[83]Arcaini L, Bruno R. Hepatitis C virus infection and antiviral treatment in marginal zone lymphomas. Curr Clin Pharmacol 2010; 5 :74-81.

[84]Carrier P, Jaccard A, Jacques J, *et al*. HCV-associated B-cell non-Hodgkin lymphomas and new direct antiviral agents. Liver Int 2015.

[85]Russi S, Sansonno L, Sansonno D. Hepatitis C virus-associated B-cell non-Hodgkin's lymphoma: clinical and therapeutic challenges. Curr Drug Targets, in press.

[86]Vassilopoulos D, Calabrese LH. Rheumatic manifestations of hepatitis C infection. Curr Rheumatol Rep 2003; 5: 200-4.

[87]Antonelli A, Ferri C, Galeazzi M, *et al*. HCV infection: pathogenesis, clinical manifestations and therapy. Clin Exp Rheumatol 2008; 26: 39-47.

[88]Sayiner ZA, Haque U, Malik MU, Gurakar A. Hepatitis C virus infection and its rheumatologic implications. Gastroenterol Hepatol (N Y) 2014; 10: 287-93.

[89]Nocturne G, Mariette X. Sjögren Syndrome-associated lymphomas: an update on pathogenesis and management. Br J Haematol 2015; 168: 317-27.

[90]Patel R, Mikuls TR, Richards JS, *et al*. Disease characteristics and treatment patterns in veterans with rheumatoid arthritis and concomitant hepatitis C infection. Arthritis Care Res (Hoboken) 2015; 67: 467-74.

[91]Wang Y, Dou H, Liu G, *et al*. Hepatitis C virus infection and the risk of Sjögren or sicca syndrome: a meta-analysis. Microbiol Immunol 2014; 58: 675-87.

[92]Ferri C, Sebastiani M, Antonelli A, Colaci M, Manfredi A, Giuggioli D. Current treatment of hepatitis C-associated rheumatic diseases. Arthritis Res Ther 2012; 14: 215.

[93] Giuggioli D, Sebastiani M, Colaci M, *et al*. *Treatment of HCV-related mixed cryoglobulinemia*. Curr Drug Targets, in press.

[94] Sebastiani M, Giuggioli D, Colaci M, *et al.* HCV-related rheumatic manifestations and therapeutic strategies. Curr Drug Targets, in press.

[95]Antonelli A, Ferri C, Fallahi P, *et al*. Thyroid disorders in chronic hepatitis C virus infection. Thyroid 2006; 16: 563-72.

[96]Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmun Rev 2015; 14: 174-80.

[97]Antonelli A, Ferri C, Fallahi P, *et al.* Thyroid cancer in HCV-related chronic hepatitis patients: a case-control study. Thyroid 2007; 17 :447-51.

[98]Antonelli A, Ferri C, Fallahi P, *et al*. Hepatitis C virus infection: evidence for an association with type 2 diabetes. Diabetes Care 2005; 28 :2548-50.