

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2021 June 14; 27(22): 2921-3141



**FRONTIER**

- 2921 Fecal microbiota transplantation for irritable bowel syndrome: An intervention for the 21<sup>st</sup> century  
*El-Salhy M, Patcharatrakul T, Gonlachanvit S*

**REVIEW**

- 2944 Interplay between nuclear factor erythroid 2-related factor 2 and inflammatory mediators in COVID-19-related liver injury  
*Zhu DD, Tan XM, Lu LQ, Yu SJ, Jian RL, Liang XF, Liao YX, Fan W, Barbier-Torres L, Yang A, Yang HP, Liu T*
- 2963 Mucosal lesions of the upper gastrointestinal tract in patients with ulcerative colitis: A review  
*Sun Y, Zhang Z, Zheng CQ, Sang LX*
- 2979 Application of artificial intelligence-driven endoscopic screening and diagnosis of gastric cancer  
*Hsiao YJ, Wen YC, Lai WY, Lin YY, Yang YP, Chien Y, Yarmishyn AA, Hwang DK, Lin TC, Chang YC, Lin TY, Chang KJ, Chiou SH, Jheng YC*
- 2994 Hepatocellular carcinoma in viral and autoimmune liver diseases: Role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment  
*Granito A, Muratori L, Lalanne C, Quarneti C, Ferri S, Guidi M, Lenzi M, Muratori P*

**MINIREVIEWS**

- 3010 Role of bile acids in liver diseases mediated by the gut microbiome  
*Shao JW, Ge TT, Chen SZ, Wang G, Yang Q, Huang CH, Xu LC, Chen Z*
- 3022 Liver injury in COVID-19: Detection, pathogenesis, and treatment  
*Cai Y, Ye LP, Song YQ, Mao XL, Wang L, Jiang YZ, Que WT, Li SW*
- 3037 Role of imaging in evaluating the response after neoadjuvant treatment for pancreatic ductal adenocarcinoma  
*Zhang Y, Huang ZX, Song B*
- 3050 Current approach to treatment of minimal hepatic encephalopathy in patients with liver cirrhosis  
*Moran S, López-Sánchez M, Milke-García MDP, Rodríguez-Leal G*
- 3064 COVID-19 and pediatric fatty liver disease: Is there interplay?  
*Di Sessa A, Lanzaro F, Zarrilli S, Picone V, Guarino S, Miraglia del Giudice E, Marzuillo P*

**ORIGINAL ARTICLE****Basic Study**

- 3073 Enhancer of zeste homolog 2 contributes to apoptosis by inactivating janus kinase 2/ signal transducer and activator of transcription signaling in inflammatory bowel disease  
*Zhou J, Yang Y, Wang YL, Zhao Y, Ye WJ, Deng SY, Lang JY, Lu S*

**Retrospective Study**

- 3085 Quinone oxidoreductase 1 is overexpressed in gastric cancer and associated with outcome of adjuvant chemotherapy and survival  
*Jiang ZN, Ahmed SMU, Wang QC, Shi HF, Tang XW*
- 3097 Idiopathic mesenteric phlebosclerosis associated with long-term oral intake of geniposide  
*Wen Y, Chen YW, Meng AH, Zhao M, Fang SH, Ma YQ*
- 3109 Early serum albumin changes in patients with ulcerative colitis treated with tacrolimus will predict clinical outcome  
*Ishida N, Miyazu T, Tamura S, Tani S, Yamade M, Iwaizumi M, Hamaya Y, Osawa S, Furuta T, Sugimoto K*

**Observational Study**

- 3121 Preservation of superior rectal artery in laparoscopically assisted subtotal colectomy with ileorectal anastomosis for slow transit constipation  
*Wu CW, Pu TW, Kang JC, Hsiao CW, Chen CY, Hu JM, Lin KH, Lin TC*

**Prospective Study**

- 3130 High fecal calprotectin levels are associated with SARS-CoV-2 intestinal shedding in COVID-19 patients: A proof-of-concept study  
*Zerbato V, Di Bella S, Giuffrè M, Jaracz AW, Gobbo Y, Luppino D, Macor P, Segat L, Koncan R, D'Agaro P, Valentini M, Crocé LS, Ruscio M, Luzzati R*

**CORRECTION**

- 3138 *Helicobacter pylori* promotes invasion and metastasis of gastric cancer by enhancing heparanase expression  
*Liu LP, Sheng XP, Shuai TK, Zhao YX, Li B, Li YM*

**ABOUT COVER**

Editorial Board Member of *World Journal of Gastroenterology*, Francisco Rodriguez-Frias, MS, PhD, Senior Investigator, Associate Professor at Sciences and Medicine Schools, Autonomia University of Barcelona; Head of Special Units (Biochemistry Department) and Head of Liver Pathology Laboratory (Biochemistry and Microbiology Departments) of Vall d'Hebron University Hospital; Head of Biochemistry Research Group or Vall d'Hebron Institute of Research, Passeig Vall d'Hebron, 119-129, Barcelona 08035, Spain. frarodri@vhebron.net

**AIMS AND SCOPE**

The primary aim of *World Journal of Gastroenterology (WJG, World J Gastroenterol)* is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

**INDEXING/ABSTRACTING**

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2020 edition of Journal Citation Report® cites the 2019 impact factor (IF) for *WJG* as 3.665; IF without journal self cites: 3.534; 5-year IF: 4.048; Ranking: 35 among 88 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2019 is 7.1 and Scopus CiteScore rank 2019: Gastroenterology is 17/137.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Production Editor: *Ji-Hong Lin*; Production Department Director: *Yun-Xiaojuan Wu*; Editorial Office Director: *Ze-Mao Gong*.

**NAME OF JOURNAL**

*World Journal of Gastroenterology*

**ISSN**

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

**LAUNCH DATE**

October 1, 1995

**FREQUENCY**

Weekly

**EDITORS-IN-CHIEF**

Andrzej S Tarnawski, Subrata Ghosh

**EDITORIAL BOARD MEMBERS**

<http://www.wjgnet.com/1007-9327/editorialboard.htm>

**PUBLICATION DATE**

June 14, 2021

**COPYRIGHT**

© 2021 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION ETHICS**

<https://www.wjgnet.com/bpg/GerInfo/288>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Hepatocellular carcinoma in viral and autoimmune liver diseases: Role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment

Alessandro Granito, Luigi Muratori, Claudine Lalanne, Chiara Quarneti, Silvia Ferri, Marcello Guidi, Marco Lenzi, Paolo Muratori

**ORCID number:** Alessandro Granito 0000-0002-0637-739X; Luigi Muratori 0000-0002-8748-8031; Claudine Lalanne 0000-0002-8438-9060; Chiara Quarneti 0000-0002-0637-731X; Silvia Ferri 0000-0002-6033-8594; Marcello Guidi 0000-0002-8857-0096; Marco Lenzi 0000-0001-8558-0793; Paolo Muratori 0000-0002-5899-5966.

**Author contributions:** Granito A, Muratori L and Muratori P reviewed the literature and drafted the manuscript; Granito A, Muratori L, Lalanne C, Ferri S, Quarneti C and Muratori P analyzed and critically interpreted literature data; all authors were involved in acquisition of data; all authors critically reviewed the manuscript and approved the final version of this manuscript.

**Conflict-of-interest statement:** All the authors have no conflict of interest to declare.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to

**Alessandro Granito**, Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, University of Bologna, Bologna 40138, Italy

**Alessandro Granito**, Center for the Study and Treatment of Autoimmune Diseases of the Liver and Biliary System, Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy

**Luigi Muratori, Marco Lenzi**, Division of Internal Medicine and Immunorheumatology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Center for the Study and Treatment of Autoimmune Diseases of the Liver and Biliary System, University of Bologna, Bologna 40138, Italy

**Luigi Muratori, Marco Lenzi**, Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy

**Claudine Lalanne, Chiara Quarneti, Silvia Ferri, Marcello Guidi**, Division of Internal Medicine, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Center for the Study and Treatment of Autoimmune Diseases of the Liver and Biliary System, Bologna 40138, Italy

**Paolo Muratori**, Division of Internal Medicine, Morgagni-Pierantoni Hospital, Forlì 47100, Italy

**Paolo Muratori**, Department of Science for the Quality of Life, University of Bologna, Bologna 40138, Italy

**Corresponding author:** Alessandro Granito, MD, Assistant Professor, Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, University of Bologna, Via Albertoni 15, Bologna 40138, Italy. [alessandro.granito@unibo.it](mailto:alessandro.granito@unibo.it)

### Abstract

More than 90% of cases of hepatocellular carcinoma (HCC) occurs in patients with cirrhosis, of which hepatitis B virus and hepatitis C virus are the leading causes, while the tumor less frequently arises in autoimmune liver diseases. Advances in understanding tumor immunity have led to a major shift in the treatment of HCC, with the emergence of immunotherapy where therapeutic agents are used to

distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Italy

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0  
Grade B (Very good): B, B, B  
Grade C (Good): 0  
Grade D (Fair): 0  
Grade E (Poor): 0

**Received:** February 4, 2021

**Peer-review started:** February 4, 2021

**First decision:** March 28, 2021

**Revised:** April 9, 2021

**Accepted:** May 7, 2021

**Article in press:** May 7, 2021

**Published online:** June 14, 2021

**P-Reviewer:** Chen W, Shen L

**S-Editor:** Gao CC

**L-Editor:** A

**P-Editor:** Liu JH



target immune cells rather than cancer cells. Regulatory T cells (Tregs) are the most abundant suppressive cells in the tumor microenvironment and their presence has been correlated with tumor progression, invasiveness, as well as metastasis. Tregs are characterized by the expression of the transcription factor Foxp3 and various mechanisms ranging from cell-to-cell contact to secretion of inhibitory molecules have been implicated in their function. Notably, Tregs amply express checkpoint molecules such as cytotoxic T lymphocyte-associated antigen 4 and programmed cell-death 1 receptor and therefore represent a direct target of immune checkpoint inhibitor (ICI) immunotherapy. Taking into consideration the critical role of Tregs in maintenance of immune homeostasis as well as avoidance of autoimmunity, it is plausible that targeting of Tregs by ICI immunotherapy results in the development of immune-related adverse events (irAEs). Since the use of ICI becomes common in oncology, with an increasing number of new ICI currently under clinical trials for cancer treatment, the occurrence of irAEs is expected to dramatically rise. Herein, we review the current literature focusing on the role of Tregs in HCC evolution taking into account their opposite etiological function in viral and autoimmune chronic liver disease, and we discuss their involvement in irAEs due to the new immunotherapies.

**Key Words:** Autoimmune liver disease; Hepatitis B virus-related chronic hepatitis; Hepatitis C virus-related chronic hepatitis; Hepatocellular carcinoma; Tumor microenvironment

©The Author(s) 2021. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Hepatocellular carcinoma (HCC) is the fifth most common cancer with poor prognosis despite significant improved diagnostic and therapeutic strategies. Most of HCC occurs in cirrhotic patients, with hepatitis B and C viruses being leading causes. Recent studies showed that HCC development and progression are associated with a unique immune response profile of the liver microenvironment where CD4+CD25+ Foxp3 regulatory T-cells (Tregs) play a crucial role through their immunosuppressive role. We discuss the role of Tregs in chronic liver diseases as well as in HCC initiation and the role of immunotherapy to enhance anti-tumor immune response by blocking Treg activity.

**Citation:** Granito A, Muratori L, Lalanne C, Quarneti C, Ferri S, Guidi M, Lenzi M, Muratori P. Hepatocellular carcinoma in viral and autoimmune liver diseases: Role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment. *World J Gastroenterol* 2021; 27(22): 2994-3009

**URL:** <https://www.wjgnet.com/1007-9327/full/v27/i22/2994.htm>

**DOI:** <https://dx.doi.org/10.3748/wjg.v27.i22.2994>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most frequent cause of cancer-related death worldwide, with more than 900000 new cases and more than 800000 deaths in 2020[1].

HCC accounts for nearly 90% of primary liver cancers and is a leading world health problem. The incidence of HCC rises sharply with age in all populations, achieving a peak at age 70 and it is increasing in most countries representing the dominant cause of mortality in cirrhotic patients[2-4].

Worldwide, chronic viral hepatitis has been reported as the leading risk factors for HCC development, although in high-income areas HCC related to non-alcoholic fatty liver disease is increasing due to the rising prevalence of metabolic disorders[5-7].

In contrast, vaccination and treatment for hepatitis B virus (HBV) infection, prevention campaigns for sexual and iatrogenic transmission of hepatitis B (HBV) and hepatitis C virus (HCV), and the introduction of effective HCV antiviral agents are reducing the burden of chronic viral liver disease[8-11].

HCC arises almost exclusively in the setting of chronic liver inflammation and, unlike the elevated risk associated with chronic viral (HBV and HCV) infections, it has been reported that the tumor is less common in liver cirrhosis caused by autoimmune liver diseases[12-15]. However, it is known that, regardless of etiology, cirrhosis *per se* represents a precancerous condition leading to an increased risk of HCC[15,16].

Indeed, irrespective of etiology, a typical sequence of chronic necroinflammation, compensatory liver regeneration, induction of liver fibrosis and subsequent cirrhosis often precedes hepatocarcinogenesis. HCC is a prototypical inflammation-driven tumor arising on the backdrop of liver cirrhosis. The evidence of an immune-rich contexture of the HCC microenvironment has inspired several studies in recent years that have further defined profile and crucial pathogenetic role of immune cells in tumor development[17].

The liver is a central immunomodulator that ensures organ and systemic protection while maintaining immunotolerance. Deregulation of this tightly controlled liver immunological network is a hallmark of chronic liver disease and HCC[18].

Recently, within the cell subset characterizing the HCC immune microenvironment, a key role has been highlighted for CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (Tregs), which are crucially implicated in both pathogenesis of chronic liver diseases and development and spread of HCC[19,20].

In this review we examine the evidence that has recently accumulated on the different role that these cells play in chronic viral liver diseases (HBV and HCV) and autoimmune liver diseases, and their function in the tumor microenvironment (TME) characterizing HCC. We also discuss the possible implications for emerging immunotherapies and the potential risks of immune-mediated liver toxicity from this treatment [21].

---

## HCC RISK IN VIRAL AND AUTOIMMUNE LIVER DISEASES

---

It is well established that cirrhosis represents the most significant risk factor for the development of HCC[22]. Historically, it has been reported that the risk of HCC in cirrhosis due to viral causes is higher than in other non-viral etiologies, however a precise comparison of the incidence of HCC in various chronic liver diseases, especially in cirrhosis, has only recently been evaluated[23,24].

In a recent meta-analysis it was shown that the annual incidence of HCC in chronic liver diseases and the ratio of HCC incidence in non-cirrhotic/cirrhotic stages has the following etiological hierarchy: HCV-related disease 0.68% to 4.81% (7.07-fold,  $P < 0.001$ ), HBV-related liver disease 0.37% to 3.23% (8.73 fold,  $P < 0.001$ ), primary biliary cholangitis (PBC) (pre-cirrhotic *vs* Scheuer's III-IV stage) 0.26 to 1.79% (6.88-fold,  $P < 0.001$ ), NASH 0.03% to 1.35% (45-fold,  $P < 0.001$ ), autoimmune hepatitis (AIH) 0.19% to 0.53% (2.79-fold,  $P = 0.03$ ), and that the incidence of HCC is markedly increased (2.79-fold to 45-fold) in the cirrhotic stage compared with the non-cirrhotic stage, regardless of etiology[24]. Thus, it is confirmed that there is a significant difference in HCC incidence between viral and autoimmune liver diseases, with the lowest risk in the latter even when in the cirrhotic stage.

Worldwide, about 54% of cases can be ascribed to HBV infection (affecting 400 million people globally) while 31% can be associated with HCV infection (affecting 170 million people), leaving about 15% attributable to other causes[25].

Incidence of HCC in autoimmune liver disease is less definitively established. In a recent systematic review of 25 published cohorts, a total of 6.528 AIH patients with a median follow-up of 8 years were evaluated for the incidence of HCC. The pooled incidence rate was 3.1 per 1.000 person-years in AIH patients that tripled in those with cirrhosis[26,27].

PBC-related cirrhosis has also been reported as a potential HCC risk factor. In a study of 273 PBC-related cirrhotic patients, followed for 3 years, the incidence rate was 5.9%, significantly higher in males with stage III/IV disease than in females[28].

In a systematic review of 17 studies, including 16.368 patients seen between 1984 and 2011, compared with the general population, PBC patients exhibited a significantly higher risk of HCC (pooled risk ratio 18.80; 95% confidence interval: 10.81-26.79)[29].

Factors associated with an increased risk of HCC development in chronic liver disease have been only partially defined. However, chronic inflammation has been reported as a crucial mechanism for the development of HCC[30-32].

In this respect, it has been reported that in HBV and HCV cirrhotic patients, transaminase serum level is one of the predictive factors for the development of HCC[32].

Similar findings have been reported in AIH as persistent elevation of serum transaminases was reported to be associated with development of HCC hence supporting the prominent pathogenic role of chronic inflammation. Other emerged risk factors included cirrhosis  $\geq 10$  years, portal hypertension, and immunosuppressive therapy  $\geq 3$  years[33].

The risk factors of HCC usually lead to a unresolving inflammatory response and necrosis resulting in tissue damage which in turn drives the sequential development of regeneration, fibrosis, cirrhosis, and eventually HCC[34,35]. In parallel, immune cells within the premalignant environment produce a wide range of cytokines, growth factors, chemokines, prostaglandins, and proangiogenic factors, contributing to an environment that supports hepatocyte transformation and promotes their survival through activation of anti-apoptotic pathways, neoangiogenesis and inhibition of immune surveillance[36].

It has been recently established that the carcinogenic process is aided by a host of immuno-related factors intrinsically linked to cell infiltrate, chemokines and their receptors that foster cell survival and proliferation[37].

In this regard, it has increasingly gained relevance to fully define the immunological characteristics of liver immune microenvironment.

## TREGS IN VIRAL AND AUTOIMMUNE LIVER DISEASES

The liver can be considered as an “immunological” organ, housing a wide range of resident immune cells performing key functions in preserving organ homeostasis[38].

As a result of its intrinsic role in detoxification, the liver is repeatedly exposed to external agents, including dietary products or commensal bacteria derived from the intestine *via* the portal vein, as well as infectious microorganisms arising from the systemic circulation *via* the arterial vein. Therefore, immune surveillance in the organ is extremely dynamic.

Resident innate immune cells comprising macrophages or Kupffer cells, natural killer (NK) cells, NKT cells, and dendritic cells (DCs) are recognized as the most predominant sentinels in the liver[39]. In addition, tissue resident memory T cells, which are normally homing cells without recirculating and which readily attack pathogens at the site of infection, are also implicated[40]. However, a key role appears to be played by resident Tregs that are highly specialized in preserving tissue tolerance [19,41]. Tregs are a subset of T lymphocytes that regulate the immune response by suppressing the proliferation and cytokines production of effector T lymphocytes[42, 43].

In 2003, the forkhead box transcription factor *foxp3* was identified as a specific marker of Tregs, and its expression was found crucial for their suppressive activity[44-46].

Tregs arise in the thymus, constitutively express high levels of the interleukin (IL)-2 receptor (IL-2R)  $\alpha$  chain (CD25), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and glucocorticoid-induced TNF receptor family-related gene (*GITR*), accounting for 5% to 10% of peripheral CD4<sup>+</sup> T cells[47,48].

They play a critical role in mediating immunological self-tolerance by suppressing self-reactive T lymphocytes[44]. Tregs have been proposed to operate both through core mechanisms of suppression, including IL-2 deprivation and CTLA-4-mediated downregulation of costimulatory molecules on antigen presenting cells (APCs), and by diverse context-dependent mechanisms, including the secretion of cytokines[49-52]. Earlier studies have confirmed that Tregs mediate suppressive effects *in vivo* mainly through the production of inhibitory cytokines such as IL-10, IL-35 and transforming growth factor  $\beta$  (TGF- $\beta$ )[53-55].

With the discovery of Tregs and the understanding of their immunosuppressive role, evidences have been accumulated that this cell population is decisively implicated in the pathogenesis of various conditions such as chronic viral and autoimmune liver diseases as well as HCC[56].

In particular, CD4<sup>+</sup>CD25<sup>+</sup> Tregs are thought to contribute to the impaired immune response during chronic HBV and HCV infection. Patients with chronic HBV infection are characterized by increased percentage of CD4<sup>+</sup>CD25<sup>+</sup> Tregs in their peripheral blood and a significant accumulation of these cells in the liver, with a positive correlation between their frequency and serum HBV DNA load[57-60]. Similarly, in patients with persistent HCV infection it has been reported an increased frequency of CD4<sup>+</sup>CD25<sup>+</sup> Tregs in the blood and in the liver[61-65]. Taken together, these data prove that chronic HBV and HCV infections are immunologically characterized by a host immune response suppression driven by Tregs.

On the contrary, autoimmune liver diseases are related to both numerical and functional defect of CD4+CD25+ Tregs, to the extent that therapeutic interventions aimed at restoring an adequate number and function of these cells are followed by a remission of the autoimmune inflammatory activity[66,67].

These findings have thus outlined a pattern of chronic inflammation characterized by a diametrically opposed liver immune phenotype in chronic viral and autoimmune diseases, the former being characterized by a predominance of Tregs exerting an immunosuppressive effect that hinders the antiviral response and infection eradication, the latter by a significant numerical and functional deficiency of Tregs that do not adequately suppress self-reactive lymphocytes[68-70].

While it has been widely reported that TME characterizing HCC during chronic viral liver disease is dominated by a marked Treg infiltration likely in continuity with the conditions favoring chronic infection, the expression pattern of Tregs in the TME supporting HCC development in autoimmune liver disease is not as well known[71, 72].

This finding would be relevant for the assessment of potential adverse events related to emerging immunotherapies that cause a decline in Treg number and function and are therefore associated with the risk of triggering autoimmune disorders, as we discuss below.

---

## IMMUNE TME IN HCC: ROLE OF CD4+CD25+ TREGS

---

Tumor infiltrating lymphocytes represent the host immune response to cancer and comprise CD8+ cytotoxic T lymphocytes (CTLs) and NK cells as favorable anti-tumor responders, and CD4+ CD25+ Tregs as immunosuppressors.

Recently, many studies have shown that the TME plays a major role in HCC initiation and progression[73]. Lymphocytes contribute to the TME through immunity and inflammation. CD8+ CTLs can directly kill target cells by releasing granules including membrane-lytic materials such as perforin and granzymes (granzyme A and B) in acquired immune responses, thus covering a crucial role in anti-tumor immunity. As a matter of fact, a large presence of CD8+ CTL infiltrating tumor tissue is closely associated with a better prognosis[74,75]. However, despite T cell infiltration, HCC develops and spreads as a result of a depletion of pro-inflammatory T cells and a significant accumulation of Tregs[76-78].

T-cell exhaustion is characterized by reduced responses to stimulation, impaired cytokine production, decreased proliferation and reduced toxicity. Such immune profile is hallmarked by over-expression of co-inhibitory receptors such as CTLA-4 and programmed cell-death 1 receptor (PD-1).

An exhausted state of circulating and intratumoral CD8+ T cells is associated with a worse prognosis in HCC patients[79,80].

Exhaustion inside the TME is dominated by the inhibitory cytokine environment rich in IL-10 and TGF- $\beta$  released by the Treg, both prohibiting CTLs and TH1 CD4+ T cells activation[81,82].

In addition, tumor associated neutrophils (by secreting CCL17 and CCR4) and M2 tumor-associated macrophages (by secreting IL-10) can induce CD4+ CD25+ Tregs thereby indirectly supporting tumor growth and progression[83,84] (Figure 1).

---

## TARGETING CD4+CD25+ TREGS IN HCC MICROENVIRONMENT WITH IMMUNOTHERAPY

---

A significant advance in the field of immunotherapy has now reached with the use of immune checkpoint inhibitors (ICIs), which are antagonistic antibodies that inhibit key immune regulatory molecules (checkpoint molecules), such as CTLA-4, PD-1, and its ligand PD-L1, which suppresses T cell effector function under physiological conditions [85].

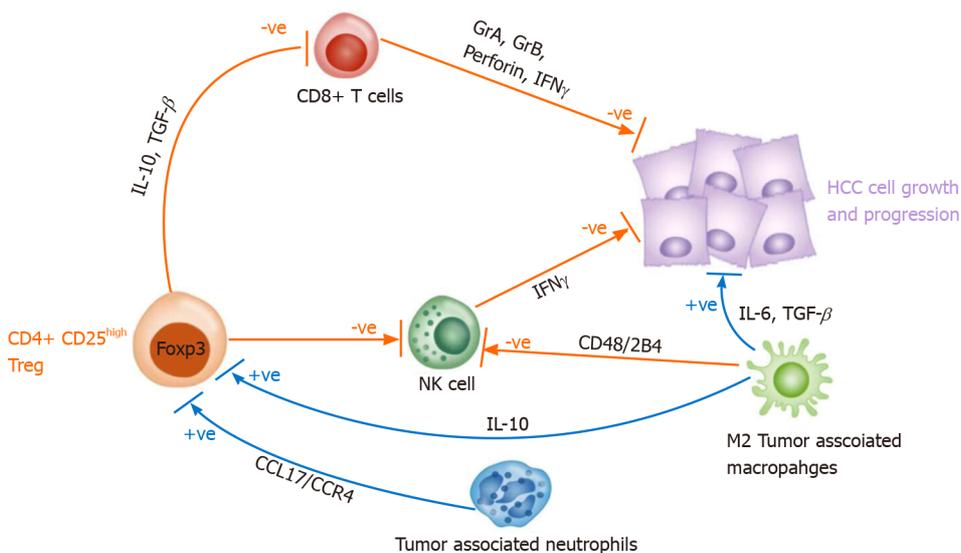
Tregs are among the most prevalent suppressor cells in TME and their presence has been related to tumor progression, invasiveness, and metastasis. Their regulatory function involves a broad spectrum of immune cells besides T cells, including macrophages, DCs, neutrophils, NK cells, T cells, and innate lymphoid cells[86-88].

There are many factors favoring Treg enrichment in the TME. Experimental evidences implicate the Treg recruitment within the tumor mass through chemokines produced by cancer cells and, specifically, HCC cells have been shown to secrete CCL5, CCL22 and CCL28 chemokines mediating Treg accumulation (Table 1)[89-100].

Table 1 Regulatory T cell function and recruitment in hepatocellular carcinoma

Treg function in HCC		Ref.
Molecule	Target	
IL-10	IL-10R	Marra and Tacke[90]
IL-35	IL-12R $\beta$ 2	Shen <i>et al</i> [91]
TGF- $\beta$	TGF- $\beta$ R	Fu <i>et al</i> [92]
CTLA-4	CD80/CD86	Chen <i>et al</i> [93]
CD39-CD73	ATP	Chen <i>et al</i> [94]
IL-2 R $\alpha$ (CD 25)	IL-2	Li <i>et al</i> [95]
LAG3	MHC class II molecules	Cabrera <i>et al</i> [96]
Treg recruitment in HCC		Ref.
Molecule	Receptor	
CCL22	CCR4	Li <i>et al</i> [97]
CCL5	CCR5	Cheng <i>et al</i> [98]
CCL28	CCR3, CCR10	Singh <i>et al</i> [99]

CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; LAG3: Lymphocyte activation gene 3 protein; IL: Interleukin; TGF- $\beta$ : Transforming growth factor  $\beta$ ; Treg: Regulatory T cell; HCC: Hepatocellular carcinoma.

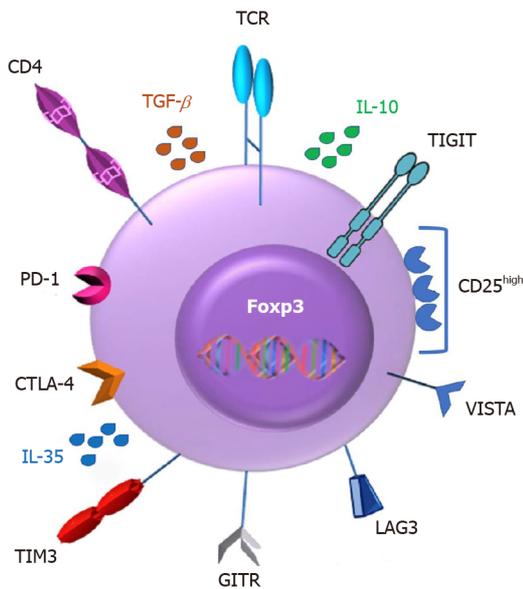


**Figure 1** The hepatocellular carcinoma liver microenvironment is characterized by a large population of immune cells. Natural killer and CD8+ T cells exert antitumor effect by secreting interferon- $\gamma$ , granzyme A, granzyme B and perforin, however they are mostly defective since these cells are suppressed by CD4+ CD25 regulatory T cell (Tregs). Additionally, tumor associated neutrophils (by secreting CCL17 and CCR4) and M2 tumor associated macrophages (by secreting interleukin-10) can induce CD4+ CD25+ Tregs thus supporting tumor growth and progression. NK: Natural killer; Treg: Regulatory T cell; IL: Interleukin; TGF- $\beta$ : Transforming growth factor  $\beta$ ; IFN: Interferon; HCC: Hepatocellular carcinoma.

The mechanisms through which Tregs induce suppression of proliferation, activation and function of immune effector cells have been well studied. Firstly, they modulate the activity of APCs by engaging inhibitory co-stimulatory receptors on their surface and in this way, signaling between APCs and T cells is impaired or abolished [101,102].

On a similar line, they down-regulate the expression of CD40, CD80 and CD86 on DCs [103]. Second, Tregs, through the secretion of inhibitory cytokines (*e.g.*, IL-10, IL-35, TGF- $\beta$ ), repress the activity of immune cells [104].

Of major interest, Tregs express a panel of chemokine receptors and surface molecules such as CTLA4, PD-1 and others, thus potentially making them a very direct target of ICI immunotherapy (Figure 2).



**Figure 2** Regulatory T cells are recognized as CD4+CD25<sup>high</sup> positive and Foxp3<sup>+</sup> expressing cells. They feature a range of other phenotypic markers such as T-cell immunoreceptor with Ig and ITIM domains, glucocorticoid-induced tumor necrosis factor receptor-related protein, cytotoxic T lymphocyte-associated antigen 4, programmed cell-death 1 receptor, V-domain Ig suppressor of T cell activation, lymphocyte activation gene-3, T cell immunoglobulin mucin 3. Upon activation, Tregs release the inhibitory cytokines interleukin (IL)-10, transforming growth factor  $\beta$  and IL-35. TCR: T-cell receptor; IL: Interleukin; TGF- $\beta$ : Transforming growth factor  $\beta$ ; TIGIT: T-cell immunoreceptor with Ig and ITIM domains; VISTA: V-domain Ig suppressor of T cell activation; LAG3: Lymphocyte activation gene-3; GITR: Glucocorticoid-induced tumor necrosis factor receptor-related protein; TIM3: T cell immunoglobulin mucin 3; CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; PD-1: Programmed cell-death 1 receptor.

Advances in understanding tumor immunity have resulted in a significant shift in the HCC treatment, with the emergence of immunotherapy where therapeutic interventions are used to target immune cells rather than cancer cells.

Tregs abundantly express both co-inhibitory and co-stimulatory molecules at levels that are likely dependent on the TME. Modulating their function through stimulation of inhibitory receptors and inhibition of activating receptors could therefore result in a decrease of the TME immunosuppressive profile resulting ultimately in an enhanced antitumor immune response (Figure 3).

In support of this therapeutic approach, treatment with blocking antibodies for PD-1 (nivolumab, pembrolizumab, sintilimab, penpulimab, camrelizumab, toripalimab, spartalizumab, tislelizumab), programmed death ligand 1 (durvalumab, avelumab, atezolizumab), and CTLA-4 (tremelimumab, ipilimumab) have reported promising results in HCC treatment[105].

Since CTLA-4 is constitutively expressed on Tregs, its specific deletion is associated with a marked reduction of their suppressive ability potentially resulting in a severe T cell mediated autoimmune disease[106].

Resulting in a break of balance in the immune system, ICI treatments can give rise to a broad spectrum of serious autoimmune manifestations, including liver injury, reported in recent studies as immune-related adverse events (irAEs) and potentially limiting or precluding HCC treatments[107,108].

Unfortunately, clinical studies that include in their objectives the assessment of immune cell populations, particularly Tregs, are still too scarce, thus leaving this potentially relevant issue unresolved. Clinical trials based on ICI agents as treatment for HCC are reported in Table 2.

## LIVER TOXICITY DURING TREATMENT WITH ICIS

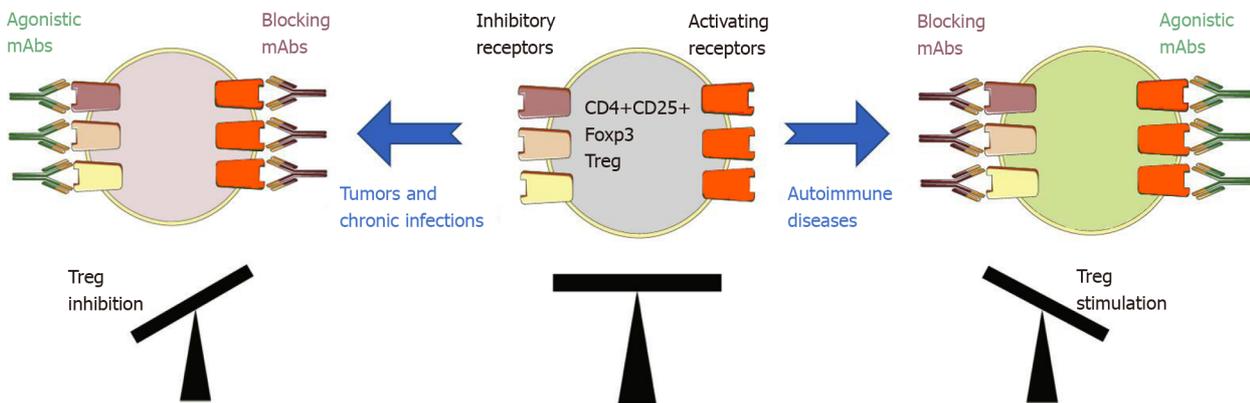
The development of irAEs, potentially affecting multiple organs, following loss of self-tolerance, has been widely reported[109,110].

Among the possible adverse events, of particular relevance is the development of liver toxicity as it could cause worsening of liver function in patients who almost always have underlying preexisting chronic liver disease[107].

**Table 2 Clinical trials assessing immune cell populations as study outcome**

Clinical trials identifier	Official title	Phase	Secondary outcome	Intervention/treatment	Status
NCT04518774	The safety assessment of <i>ex vivo</i> expanded allogeneic $\gamma\delta$ T cells in hepatocellular carcinoma patients in phase 1 clinical trial	I	Number and phenotype of $\gamma\delta$ T cells in peripheral blood	Patients will receive 3 cycles of <i>ex vivo</i> expanded allogeneic $\gamma\delta$ T cells treatments, at four-weeks' intervals, each cycle has 2 infusions. <i>Ex vivo</i> expanded $\gamma\delta$ T cells are transfused to patients in a dosage escalated manner (dose escalation, $1 \times 10^7$ , $3 \times 10^7$ , $9 \times 10^7$ per kg of body weight)	Recruiting
NCT03841201	IMMUNIB: An open-label, single-arm phase II study of immunotherapy with nivolumab in combination with lenvatinib for advanced stage	II	Immune cell infiltrates FOXP3 expression	Lenvatinib peroral qd (8 mg for patients with body weight < 60 kg and 12 mg for patients with body weight $\geq$ 60 kg); Nivolumab i.v. q2w (240 mg fixed dose IV) max. 36 cycles	Recruiting
NCT04777708	Pilot feasibility study of intratumoral BO-112 in combination with pembrolizumab for advanced hepatocellular carcinoma	I	Intratumoral CD4+, CD8+ expression and cluster of differentiation 56 (CD56+) expression (natural killer cells)	Patients receive pembrolizumab IV over 30 min on day 1 of odd number cycles. Patients also receive BO-112 by intratumoral injection on day 1, 8, and 15 of cycle 1, and day 15 of subsequent cycles. Treatment repeats every 3 wk for up to 17 cycles in the absence of disease progression or unacceptable toxicity	Not yet recruiting
NCT04721132	An open-label, phase II, pre-operative study of atezolizumab plus bevacizumab for resectable hepatocellular carcinoma	II	To measure baseline and longitudinal changes of immune infiltration including CD8/regulatory T cell ratio and CD68+ density, and fibrosis stage	Patients receive atezolizumab IV over 30-60 min and bevacizumab IV over 30-90 min on day 1. Treatment repeats every 21 d for up to 3 cycles in the absence of disease progression or unacceptable toxicity. Patients then undergo surgery during week 12	Recruiting
NCT00396682	Elimination of CD4+CD25+ regulatory T cells in patients with advanced hepatocellular carcinoma after treatment with cyclophosphamide	I	Function and Phenotype of CD4+CD25+ regulatory T cells	Cyclophosphamide 150 mg to 250 mg to 350 mg	Completed

Clinical trials assessing as secondary study outcomes different immune cell populations, including CD4+CD25+ Tregs (<https://www.clinicaltrials.gov/>).



**Figure 3 Distinct targeting of regulatory T cell activating and inhibitory receptor-targeted therapies in cancer and autoimmunity.** Regulatory T cell (Treg) cells are equipped with a repertoire of activating and inhibitory receptors. For successful therapy of tumors and chronic infections, blockade of activating receptors and/or stimulation of inhibitory receptors shifts the balance toward inhibition of Tregs. In contrast, to achieve Treg activation in autoimmune diseases, blockade of inhibitory receptors and/or stimulation of activating receptors may be desirable. Treg: Regulatory T cell.

Hepatic toxicity associated with ICI is characterized by elevation of liver parameters values. The pattern of liver enzymes elevation is defined by the increase of alanine aminotransferase (ALT) or alkaline phosphatase (ALP) alone above a specific threshold or by the ratio of serum ALT to ALP levels [R value = [ALT/upper normal level (UNL)]/[ALP/UNL]] and can be categorized as hepatocellular (ALT  $\geq$  5-fold above UNL or R > 5), mixed (R > 2 to < 5), or cholestatic (ALP  $\geq$  2 fold above UNL or R < 2)[111].

The pattern of ICI-related liver toxicity is heterogenous since it may be cytolytic, cholestatic or mixed, although ICI-related cholestasis seems to be rarer[107].

In HCC patients receiving ICIs the incidence of liver toxicity ranges according to the type of drug and the dose received. It has been reported that liver toxicity is more frequent in HCC patients receiving anti-CTLA-4 therapies. In HCC patients receiving the anti-PD-1 antibody nivolumab (CHECKMATE 040 trial) and in those receiving another anti-PD-1 antibody pembrolizumab (KEYNOTE-224 trial), ALT elevation of any grade and of grade  $\geq 3$  was found in 15% and 6% (nivolumab) *vs* 9% and 4% (pembrolizumab), respectively[112,113].

Differently, therapy with the anti-CTLA-4 antibody tremelimumab was associated with an ALT elevation of any grade and of grade  $\geq 3$  in 19% and 9% of patients, respectively[114].

Interestingly, during HCC clinical studies, HCV and HBV positive patients exhibited a reduction in viral load during immunotherapy, more pronounced with anti-CTLA-4 agents, thus suggesting that the treatment induced immunological shift has favorable effect on antiviral response[112-116].

No definitive data has been reported about patients with autoimmune liver diseases treated with ICIs. However, in most of the HCC clinical trial, pre-existing autoimmune diseases was a contraindication for enrollment in light of previous data demonstrating that immunotherapies might trigger a flare-up of pre-existing autoimmune disease or the onset of additional immune-related disease[117-119].

No data are available concerning genetic and autoantibody profile of patients before starting ICI treatments, however autoantibodies such as antinuclear and anti-smooth muscle have been reported in patients after ICI-induced liver toxicity onset.

A management protocol for patients experiencing liver toxicity due to ICI treatment administered for non-liver tumors has been proposed and is based on corticosteroids or mycophenolate mofetil/tacrolimus (for patients not improving under corticosteroids), and ursodeoxycholic acid for those with a predominant cholestasis[120].

---

## CONCLUSION

---

Increasing understanding of the immunologic mechanisms that characterize the TME of HCC has led to a better insight into the pathogenesis of HCC and its link to chronic inflammation and cirrhosis. As well as, the different prevalence in viral *vs* non-viral liver diseases, particularly autoimmune, confirms the key role of the liver immune microenvironment.

Although the precise pathogenetic mechanisms of irAEs remain largely undefined, several processes have been proposed to be involved in the development of irAEs such as genetic factors, gut microbiome, epitope spreading, and cross-presentation of neoantigens[121,122]. A thorough evaluation on the role of Tregs in the pathogenesis of irAEs is crucial. Since cancer and autoimmunity constitute two sides of the same coin, it is perhaps not surprising that when we manipulate the immune system to treat cancer through the use of checkpoint therapy, we inevitably unbalance the vital mechanisms that regulate self-tolerance, inducing a number of irAEs. This is, at least in part, related to the impairment of Treg homeostasis, which is crucial for maintaining immune tolerance[123].

Currently used ICIs may also target Tregs, since several checkpoint molecules including CTLA4 and PD-1 are highly expressed on their surface, therefore it is possible that the development of irAEs may be in part attributed to the Treg destabilization. Consistent with this line, it has been demonstrated that anti-CTLA4 disrupts the crosstalk between Foxp3 Tregs and antigen-presenting cells to promote autoimmunity[124]. In light of the effects of immunotherapy on the enhancement of the immune response, it should be investigated whether in cases of HCC occurring in patients with pre-existing autoimmune liver and non-liver diseases these therapies are safe and not potentially hepatotoxic, since presently this information is lacking due to the exclusion of patients with autoimmune diseases from clinical trials. Of interest, other approaches to target the immunosuppressive effect of Tregs are ongoing. Given the TGF- $\beta$  mediated inhibitory role in HCC development and progression, studies are ongoing to assess how to target TGF- $\beta$ . Galunisertib, an inhibitor of TGF- $\beta$ , is currently in a phase II clinical trial for HCC patients[125,126].

Due to the potential effect of immunotherapies on Tregs and the possible effect of triggering autoimmune disorders, it is desirable that in future HCC trials the autoantibody profile, as well as genetic background (HLA) and change in the percentage of peripheral Treg during therapy be monitored to assess whether these

variables predict the risk of hepatotoxicity or extrahepatic autoimmune disorders development.

## REFERENCES

- 1 **International Agency for Research on Cancer.** Global Cancer Observatory. [cited 10 February 2021]. In: International Agency for Research on Cancer [Internet]. Available from: <https://gco.iarc.fr/today/home>
- 2 **El-Serag HB.** Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273. e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
- 3 **White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB.** Incidence of Hepatocellular Carcinoma in All 50 United States, From 2000 Through 2012. *Gastroenterology* 2017; **152**: 812-820. e5 [PMID: 27889576 DOI: 10.1053/j.gastro.2016.11.020]
- 4 **Singal AG, Lampertico P, Nahon P.** Epidemiology and surveillance for hepatocellular carcinoma: New trends. *J Hepatol* 2020; **72**: 250-261 [PMID: 31954490 DOI: 10.1016/j.jhep.2019.08.025]
- 5 **Siegel AB, Zhu AX.** Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer* 2009; **115**: 5651-5661 [PMID: 19834957 DOI: 10.1002/cncr.24687]
- 6 **Baffy G, Brunt EM, Caldwell SH.** Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.jhep.2011.10.027]
- 7 **Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, Das D, Aslam T, Patanwala I, Gaggar S, Cole M, Sumpter K, Stewart S, Rose J, Hudson M, Manas D, Reeves HL.** Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014; **60**: 110-117 [PMID: 23978719 DOI: 10.1016/j.jhep.2013.08.011]
- 8 **Singal AK, Singh A, Jaganmohan S, Guturu P, Mummadi R, Kuo YF, Sood GK.** Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *Clin Gastroenterol Hepatol* 2010; **8**: 192-199 [PMID: 19879972 DOI: 10.1016/j.cgh.2009.10.026]
- 9 **Morgan TR, Ghany MG, Kim HY, Snow KK, Shiffman ML, De Santo JL, Lee WM, Di Bisceglie AM, Bonkovsky HL, Dienstag JL, Morishima C, Lindsay KL, Lok AS; HALT-C Trial Group.** Outcome of sustained virological responders with histologically advanced chronic hepatitis C. *Hepatology* 2010; **52**: 833-844 [PMID: 20564351 DOI: 10.1002/hep.23744]
- 10 **Santi V, Buccione D, Di Micoli A, Fatti G, Frigerio M, Farinati F, Del Poggio P, Rapaccini G, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Giannini EG, Caturelli E, Chiamonte M, Bernardi M, Trevisani F.** The changing scenario of hepatocellular carcinoma over the last two decades in Italy. *J Hepatol* 2012; **56**: 397-405 [PMID: 21756850 DOI: 10.1016/j.jhep.2011.05.026]
- 11 **Simmons B, Saleem J, Heath K, Cooke GS, Hill A.** Long-Term Treatment Outcomes of Patients Infected With Hepatitis C Virus: A Systematic Review and Meta-analysis of the Survival Benefit of Achieving a Sustained Virological Response. *Clin Infect Dis* 2015; **61**: 730-740 [PMID: 25987643 DOI: 10.1093/cid/civ396]
- 12 **Rigopoulou EI, Dalekos GN.** Current Trends and Characteristics of Hepatocellular Carcinoma in Patients with Autoimmune Liver Diseases. *Cancers (Basel)* 2021; **13** [PMID: 33804480 DOI: 10.3390/cancers13051023]
- 13 **Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A.** Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
- 14 **Levrero M, Zucman-Rossi J.** Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 2016; **64**: S84-S101 [PMID: 27084040 DOI: 10.1016/j.jhep.2016.02.021]
- 15 **Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK.** Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]
- 16 **European Association for the Study of the Liver.** EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]
- 17 **Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M.** The immunology of hepatocellular carcinoma. *Nat Immunol* 2018; **19**: 222-232 [PMID: 29379119 DOI: 10.1038/s41590-018-0044-z]
- 18 **Jenne CN, Kubes P.** Immune surveillance by the liver. *Nat Immunol* 2013; **14**: 996-1006 [PMID: 24048121 DOI: 10.1038/ni.2691]
- 19 **Oo YH, Sakaguchi S.** Regulatory T-cell directed therapies in liver diseases. *J Hepatol* 2013; **59**: 1127-1134 [PMID: 23727305 DOI: 10.1016/j.jhep.2013.05.034]
- 20 **Granito A, Muratori P, Ferri S, Pappas G, Quarneti C, Lenzi M, Bianchi FB, Muratori L.** Diagnosis and therapy of autoimmune hepatitis. *Mini Rev Med Chem* 2009; **9**: 847-860 [PMID: 19519509 DOI: 10.2174/138955709788452676]
- 21 **Kole C, Charalampakis N, Tsakatikas S, Vailas M, Moris D, Gkotsis E, Kykalos S, Karamouzis MV, Schizas D.** Immunotherapy for Hepatocellular Carcinoma: A 2021 Update. *Cancers (Basel)* 2020; **12** [PMID: 33020428 DOI: 10.3390/cancers12102859]
- 22 **Fattovich G, Stroffolini T, Zagni I, Donato F.** Hepatocellular carcinoma in cirrhosis: incidence and

- risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]
- 23 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]
- 24 **Tarao K**, Nozaki A, Ikeda T, Sato A, Komatsu H, Komatsu T, Taguri M, Tanaka K. Real impact of liver cirrhosis on the development of hepatocellular carcinoma in various liver diseases-meta-analytic assessment. *Cancer Med* 2019; **8**: 1054-1065 [PMID: 30791221 DOI: 10.1002/cam4.1998]
- 25 **Global Burden of Disease Liver Cancer Collaboration**, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaean A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017; **3**: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]
- 26 **Tansel A**, Katz LH, El-Serag HB, Thrift AP, Parepally M, Shakhatreh MH, Kanwal F. Incidence and Determinants of Hepatocellular Carcinoma in Autoimmune Hepatitis: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 1207-1217. e4 [PMID: 28215616 DOI: 10.1016/j.cgh.2017.02.006]
- 27 **Lleo A**, de Boer YS, Liberal R, Colombo M. The risk of liver cancer in autoimmune liver diseases. *Ther Adv Med Oncol* 2019; **11**: 1758835919861914 [PMID: 31320937 DOI: 10.1177/1758835919861914]
- 28 **Jones DE**, Metcalf JV, Collier JD, Bassendine MF, James OF. Hepatocellular carcinoma in primary biliary cirrhosis and its impact on outcomes. *Hepatology* 1997; **26**: 1138-1142 [PMID: 9362353 DOI: 10.1002/hep.510260508]
- 29 **Liang Y**, Yang Z, Zhong R. Primary biliary cirrhosis and cancer risk: a systematic review and meta-analysis. *Hepatology* 2012; **56**: 1409-1417 [PMID: 22504852 DOI: 10.1002/hep.25788]
- 30 **Tarao K**, Rino Y, Ohkawa S, Shimizu A, Tamai S, Miyakawa K, Aoki H, Imada T, Shindo K, Okamoto N, Totsuka S. Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* 1999; **86**: 589-595 [PMID: 10440686 DOI: 10.1002/(sici)1097-0142(19990815)86:4<589::aid-cncr7>3.0.co;2-k]
- 31 **Chen CF**, Lee WC, Yang HI, Chang HC, Jen CL, Iloeje UH, Su J, Hsiao CK, Wang LY, You SL, Lu SN, Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV (REVEAL-HBV) Study Group. Changes in serum levels of HBV DNA and alanine aminotransferase determine risk for hepatocellular carcinoma. *Gastroenterology* 2011; **141**: 1240-1248. e1-1248. e2 [PMID: 21703214 DOI: 10.1053/j.gastro.2011.06.036]
- 32 **Ishikawa T**, Ichida T, Yamagiwa S, Sugahara S, Uehara K, Okoshi S, Asakura H. High viral loads, serum alanine aminotransferase and gender are predictive factors for the development of hepatocellular carcinoma from viral compensated liver cirrhosis. *J Gastroenterol Hepatol* 2001; **16**: 1274-1281 [PMID: 11903747 DOI: 10.1046/j.1440-1746.2001.02616.x]
- 33 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Predictive factors for hepatocellular carcinoma in type I autoimmune hepatitis. *Am J Gastroenterol* 2008; **103**: 1944-1951 [PMID: 18564111 DOI: 10.1111/j.1572-0241.2008.01922.x]
- 34 **Shi JH**, Line PD. Effect of liver regeneration on malignant hepatic tumors. *World J Gastroenterol* 2014; **20**: 16167-16177 [PMID: 25473170 DOI: 10.3748/wjg.v20.i43.16167]
- 35 **Li H**, Zhang L. Liver regeneration microenvironment of hepatocellular carcinoma for prevention and therapy. *Oncotarget* 2017; **8**: 1805-1813 [PMID: 27655683 DOI: 10.18632/oncotarget.12101]
- 36 **Yu LX**, Ling Y, Wang HY. Role of nonresolving inflammation in hepatocellular carcinoma development and progression. *NPJ Precis Oncol* 2018; **2**: 6 [PMID: 29872724 DOI: 10.1038/s41698-018-0048-z]
- 37 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867 [PMID: 12490959 DOI: 10.1038/nature01322]
- 38 **Ficht X**, Iannacone M. Immune surveillance of the liver by T cells. *Sci Immunol* 2020; **5**: eaba2351 [PMID: 32887842 DOI: 10.1126/sciimmunol.aba2351]
- 39 **Lian M**, Selmi C, Gershwin ME, Ma X. Myeloid Cells and Chronic Liver Disease: a Comprehensive Review. *Clin Rev Allergy Immunol* 2018; **54**: 307-317 [PMID: 29313221 DOI: 10.1007/s12016-017-8664-x]
- 40 **Pallett LJ**, Davies J, Colbeck EJ, Robertson F, Hansi N, Easom NJW, Burton AR, Stegmann KA, Schurich A, Swadling L, Gill US, Male V, Luong T, Gander A, Davidson BR, Kennedy PTF, Maini MK. IL-2<sup>high</sup> tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. *J Exp Med* 2017; **214**: 1567-1580 [PMID: 28526759 DOI: 10.1084/jem.20162115]
- 41 **Piconese S**, Cammarata I, Barnaba V. Viral hepatitis, inflammation, and cancer: A lesson for

- autoimmunity. *J Autoimmun* 2018; **95**: 58-68 [PMID: 30509387 DOI: 10.1016/j.jaut.2018.10.021]
- 42 **Thornton AM**, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**: 287-296 [PMID: 9670041 DOI: 10.1084/jem.188.2.287]
- 43 **Ng WF**, Duggan PJ, Ponchel F, Matarese G, Lombardi G, Edwards AD, Isaacs JD, Lechler RI. Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. *Blood* 2001; **98**: 2736-2744 [PMID: 11675346 DOI: 10.1182/blood.v98.9.2736]
- 44 **Fontenot JD**, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; **4**: 330-336 [PMID: 12612578 DOI: 10.1038/ni904]
- 45 **Hori S**, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057-1061 [PMID: 12522256 DOI: 10.1126/science.1079490]
- 46 **Khattri R**, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 2003; **4**: 337-342
- 47 **McHugh RS**, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002; **16**: 311-323 [PMID: 11869690 DOI: 10.1016/s1074-7613(02)00280-7]
- 48 **Shimizu J**, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 2002; **3**: 135-142 [PMID: 11812990 DOI: 10.1038/ni759]
- 49 **Wing JB**, Sakaguchi S. Multiple treg suppressive modules and their adaptability. *Front Immunol* 2012; **3**: 178 [PMID: 22754556 DOI: 10.3389/fimmu.2012.00178]
- 50 **Oderup C**, Cederbom L, Makowska A, Cilio CM, Ivars F. Cytotoxic T lymphocyte antigen-4-dependent down-modulation of costimulatory molecules on dendritic cells in CD4+ CD25+ regulatory T-cell-mediated suppression. *Immunology* 2006; **118**: 240-249 [PMID: 16771859 DOI: 10.1111/j.1365-2567.2006.02362.x]
- 51 **Onishi Y**, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells *in vitro* and actively inhibit their maturation. *Proc Natl Acad Sci U S A* 2008; **105**: 10113-10118 [PMID: 18635688 DOI: 10.1073/pnas.0711106105]
- 52 **von Boehmer H**. Mechanisms of suppression by suppressor T cells. *Nat Immunol* 2005; **6**: 338-344 [PMID: 15785759 DOI: 10.1038/ni1180]
- 53 **Izcue A**, Coombes JL, Powrie F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol Rev* 2006; **212**: 256-271 [PMID: 16903919 DOI: 10.1111/j.0105-2896.2006.00423.x]
- 54 **Zou W**. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol* 2006; **6**: 295-307 [PMID: 16557261 DOI: 10.1038/nri1806]
- 55 **Chen X**, Du Y, Lin X, Qian Y, Zhou T, Huang Z. CD4+CD25+ regulatory T cells in tumor immunity. *Int Immunopharmacol* 2016; **34**: 244-249 [PMID: 26994448 DOI: 10.1016/j.intimp.2016.03.009]
- 56 **Zheng MH**, Gu DN, Braddock M, Leishman AJ, Jin C, Wen JS, Gong YW, Chen YP. CD4+CD25+ regulatory T cells: a therapeutic target for liver diseases. *Expert Opin Ther Targets* 2008; **12**: 313-326 [PMID: 18269341 DOI: 10.1517/14728222.12.3.313]
- 57 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573 DOI: 10.4049/jimmunol.177.1.739]
- 58 **Stoop JN**, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, Janssen HL. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* 2005; **41**: 771-778 [PMID: 15791617 DOI: 10.1002/hep.20649]
- 59 **Yang G**, Liu A, Xie Q, Guo TB, Wan B, Zhou B, Zhang JZ. Association of CD4+CD25+Foxp3+ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. *Int Immunol* 2007; **19**: 133-140 [PMID: 17182968 DOI: 10.1093/intimm/dx1130]
- 60 **Peng G**, Li S, Wu W, Sun Z, Chen Y, Chen Z. Circulating CD4+ CD25+ regulatory T cells correlate with chronic hepatitis B infection. *Immunology* 2008; **123**: 57-65 [PMID: 17764450 DOI: 10.1111/j.1365-2567.2007.02691.x]
- 61 **Sugimoto K**, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. Suppression of HCV-specific T cells without differential hierarchy demonstrated *ex vivo* in persistent HCV infection. *Hepatology* 2003; **38**: 1437-1448 [PMID: 14647055 DOI: 10.1016/j.hep.2003.09.026]
- 62 **Cabrera R**, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, Nelson DR. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; **40**: 1062-1071 [PMID: 15486925 DOI: 10.1002/hep.20454]
- 63 **Boettler T**, Spangenberg HC, Neumann-Haefelin C, Panther E, Urbani S, Ferrari C, Blum HE, von Weizsäcker F, Thimme R. T cells with a CD4+CD25+ regulatory phenotype suppress *in vitro* proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 2005; **79**: 7860-7867 [PMID: 15919940 DOI: 10.1128/JVI.79.12.7860-7867.2005]
- 64 **Rushbrook SM**, Ward SM, Unitt E, Vowler SL, Lucas M, Klenerman P, Alexander GJ. Regulatory T cells suppress *in vitro* proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. *J Virol* 2005; **79**: 7852-7859 [PMID: 15919939 DOI: 10.1128/JVI.79.12.7852-7859.2005]

- 10.1128/JVI.79.12.7852-7859.2005]
- 65 **Granito A**, Maldini MC, Muratori P, Chiesa D, Bassi M *et al* Increased percentage of CD4+CD25high T cells in chronic hepatitis C patients correlates with higher viremia levels. *J Hepatol* 2005; 42: 158
  - 66 **Ferri S**, Longhi MS, De Molo C, Lalanne C, Muratori P, Granito A, Hussain MJ, Ma Y, Lenzi M, Mieli-Vergani G, Bianchi FB, Vergani D, Muratori L. A multifaceted imbalance of T cells with regulatory function characterizes type 1 autoimmune hepatitis. *Hepatology* 2010; 52: 999-1007 [PMID: 20683931 DOI: 10.1002/hep.23792]
  - 67 **Longhi MS**, Meda F, Wang P, Samyn M, Mieli-Vergani G, Vergani D, Ma Y. Expansion and de novo generation of potentially therapeutic regulatory T cells in patients with autoimmune hepatitis. *Hepatology* 2008; 47: 581-591 [PMID: 18220288 DOI: 10.1002/hep.22071]
  - 68 **Bonilla CM**, McGrath NA, Fu J, Xie C. Immunotherapy of hepatocellular carcinoma with infection of hepatitis B or C virus. *Hepatoma Res* 2020; 6 [PMID: 33134550 DOI: 10.20517/2394-5079.2020.58]
  - 69 **Chaoul N**, Mancarella S, Lupo L, Giannelli G, Dituri F. Impaired Anti-Tumor T cell Response in Hepatocellular Carcinoma. *Cancers (Basel)* 2020; 12 [PMID: 32182707 DOI: 10.3390/cancers12030627]
  - 70 **Lapierre P**, Lamarre A. Regulatory T Cells in Autoimmune and Viral Chronic Hepatitis. *J Immunol Res* 2015; 2015: 479703 [PMID: 26106627 DOI: 10.1155/2015/479703]
  - 71 **Kobayashi N**, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res* 2007; 13: 902-911 [PMID: 17289884 DOI: 10.1158/1078-0432.CCR-06-2363]
  - 72 **Kurebayashi Y**, Kubota N, Sakamoto M. Immune microenvironment of hepatocellular carcinoma, intrahepatic cholangiocarcinoma and liver metastasis of colorectal adenocarcinoma: Relationship with histopathological and molecular classifications. *Hepatol Res* 2021; 51: 5-18 [PMID: 32573056 DOI: 10.1111/hepr.13539]
  - 73 **Bian J**, Lin J, Long J, Yang X, Lu X, Sang X, Zhao H. T lymphocytes in hepatocellular carcinoma immune microenvironment: insights into human immunology and immunotherapy. *Am J Cancer Res* 2020; 10: 4585-4606 [PMID: 33415021]
  - 74 **Wada Y**, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology* 1998; 27: 407-414 [PMID: 9462638 DOI: 10.1002/hep.510270214]
  - 75 **Ding W**, Xu X, Qian Y, Xue W, Wang Y, Du J, Jin L, Tan Y. Prognostic value of tumor-infiltrating lymphocytes in hepatocellular carcinoma: A meta-analysis. *Medicine (Baltimore)* 2018; 97: e13301 [PMID: 30557978 DOI: 10.1097/MD.00000000000013301]
  - 76 **Chiou SH**, Sheu BC, Chang WC, Huang SC, Hong-Nerng H. Current concepts of tumor-infiltrating lymphocytes in human malignancies. *J Reprod Immunol* 2005; 67: 35-50 [PMID: 16111767 DOI: 10.1016/j.jri.2005.06.002]
  - 77 **Gao Q**, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; 25: 2586-2593 [PMID: 17577038 DOI: 10.1200/JCO.2006.09.4565]
  - 78 **Unitt E**, Rushbrook SM, Marshall A, Davies S, Gibbs P, Morris LS, Coleman N, Alexander GJ. Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. *Hepatology* 2005; 41: 722-730 [PMID: 15791620 DOI: 10.1002/hep.20644]
  - 79 **Ma J**, Zheng B, Goswami S, Meng L, Zhang D, Cao C, Li T, Zhu F, Ma L, Zhang Z, Zhang S, Duan M, Chen Q, Gao Q, Zhang X. PD1<sup>hi</sup> CD8<sup>+</sup> T cells correlate with exhausted signature and poor clinical outcome in hepatocellular carcinoma. *J Immunother Cancer* 2019; 7: 331 [PMID: 31783783 DOI: 10.1186/s40425-019-0814-7]
  - 80 **Tauber C**, Schultheiss M, Luca R, Buettner N, Llewellyn-Lacey S, Emmerich F, Zehe S, Price DA, Neumann-Haefelin C, Schmitt-Graeff A, Hofmann M, Thimme R. Inefficient induction of circulating TAA-specific CD8+ T-cell responses in hepatocellular carcinoma. *Oncotarget* 2019; 10: 5194-5206 [PMID: 31497249 DOI: 10.18632/oncotarget.27146]
  - 81 **Bunt SK**, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res* 2007; 67: 10019-10026 [PMID: 17942936 DOI: 10.1158/0008-5472.CAN-07-2354]
  - 82 **Vignali DA**, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol* 2008; 8: 523-532 [PMID: 18566595 DOI: 10.1038/nri2343]
  - 83 **Zhou SL**, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, Fan J, Cao Y, Dai Z, Zhou J. Tumor-Associated Neutrophils Recruit Macrophages and T-Regulatory Cells to Promote Progression of Hepatocellular Carcinoma and Resistance to Sorafenib. *Gastroenterology* 2016; 150: 1646-1658. e17 [PMID: 26924089 DOI: 10.1053/j.gastro.2016.02.040]
  - 84 **Zhou J**, Ding T, Pan W, Zhu LY, Li L, Zheng L. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. *Int J Cancer* 2009; 125: 1640-1648 [PMID: 19569243 DOI: 10.1002/ijc.24556]
  - 85 **Tovoli F**, De Lorenzo S, Trevisani F. Immunotherapy with Checkpoint Inhibitors for Hepatocellular Carcinoma: Where Are We Now? *Vaccines (Basel)* 2020; 8 [PMID: 33023131 DOI: 10.3390/vaccines8040578]
  - 86 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell*

- 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]
- 87 **Tanaka A**, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* 2017; **27**: 109-118 [PMID: 27995907 DOI: 10.1038/cr.2016.151]
- 88 **Pedroza-Pacheco I**, Madrigal A, Saudemont A. Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy. *Cell Mol Immunol* 2013; **10**: 222-229 [PMID: 23524654 DOI: 10.1038/cmi.2013.2]
- 89 **Ren L**, Yu Y, Wang L, Zhu Z, Lu R, Yao Z. Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. *Oncotarget* 2016; **7**: 75763-75773 [PMID: 27716621 DOI: 10.18632/oncotarget.12409]
- 90 **Marra F**, Tacke F. Roles for chemokines in liver disease. *Gastroenterology* 2014; **147**: 577-594. e1 [PMID: 25066692 DOI: 10.1053/j.gastro.2014.06.043]
- 91 **Shen X**, Li N, Li H, Zhang T, Wang F, Li Q. Increased prevalence of regulatory T cells in the tumor microenvironment and its correlation with TNM stage of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2010; **136**: 1745-1754 [PMID: 20221638 DOI: 10.1007/s00432-010-0833-8]
- 92 **Fu YP**, Yi Y, Cai XY, Sun J, Ni XC, He HW, Wang JX, Lu ZF, Huang JL, Cao Y, Zhou J, Fan J, Qiu SJ. Overexpression of interleukin-35 associates with hepatocellular carcinoma aggressiveness and recurrence after curative resection. *Br J Cancer* 2016; **114**: 767-776 [PMID: 27002937 DOI: 10.1038/bjc.2016.47]
- 93 **Chen J**, Gingold JA, Su X. Immunomodulatory TGF- $\beta$  Signaling in Hepatocellular Carcinoma. *Trends Mol Med* 2019; **25**: 1010-1023 [PMID: 31353124 DOI: 10.1016/j.molmed.2019.06.007]
- 94 **Chen X**, Du Y, Hu Q, Huang Z. Tumor-derived CD4+CD25+regulatory T cells inhibit dendritic cells function by CTLA-4. *Pathol Res Pract* 2017; **213**: 245-249 [PMID: 28214198 DOI: 10.1016/j.prp.2016.12.008]
- 95 **Li C**, Jiang P, Wei S, Xu X, Wang J. Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. *Mol Cancer* 2020; **19**: 116 [PMID: 32680511 DOI: 10.1186/s12943-020-01234-1]
- 96 **Cabrera R**, Ararat M, Eksioğlu EA, Cao M, Xu Y, Wasserfall C, Atkinson MA, Liu C, Nelson DR. Influence of serum and soluble CD25 (sCD25) on regulatory and effector T-cell function in hepatocellular carcinoma. *Scand J Immunol* 2010; **72**: 293-301 [PMID: 20883314 DOI: 10.1111/j.1365-3083.2010.02427.x]
- 97 **Li FJ**, Zhang Y, Jin GX, Yao L, Wu DQ. Expression of LAG-3 is coincident with the impaired effector function of HBV-specific CD8(+) T cell in HCC patients. *Immunol Lett* 2013; **150**: 116-122 [PMID: 23261718 DOI: 10.1016/j.imlet.2012.12.004]
- 98 **Cheng X**, Wu H, Jin ZJ, Ma D, Yuen S, Jing XQ, Shi MM, Shen BY, Peng CH, Zhao R, Qiu WH. Up-regulation of chemokine receptor CCR4 is associated with Human Hepatocellular Carcinoma malignant behavior. *Sci Rep* 2017; **7**: 12362 [PMID: 28959024 DOI: 10.1038/s41598-017-10267-4]
- 99 **Singh SK**, Mishra MK, Rivers BM, Gordetsky JB, Bae S, Singh R. Biological and Clinical Significance of the CCR5/CCL5 Axis in Hepatocellular Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 32260550 DOI: 10.3390/cancers12040883]
- 100 **Xue D**, Zheng Y, Wen J, Han J, Tuo H, Liu Y, Peng Y. Role of chemokines in hepatocellular carcinoma (Review). *Oncol Rep* 2021; **45**: 809-823 [PMID: 33650640 DOI: 10.3892/or.2020.7906]
- 101 **Caridade M**, Graca L, Ribeiro RM. Mechanisms Underlying CD4+ Treg Immune Regulation in the Adult: From Experiments to Models. *Front Immunol* 2013; **4**: 378 [PMID: 24302924 DOI: 10.3389/fimmu.2013.00378]
- 102 **Maj T**, Wang W, Crespo J, Zhang H, Wei S, Zhao L, Vatan L, Shao I, Szeliga W, Lyssiotis C, Liu JR, Kryczek I, Zou W. Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor. *Nat Immunol* 2017; **18**: 1332-1341 [PMID: 29083399 DOI: 10.1038/ni.3868]
- 103 **Larmonier N**, Marron M, Zeng Y, Cantrell J, Romanoski A, Sepassi M, Thompson S, Chen X, Andreansky S, Katsanis E. Tumor-derived CD4(+)CD25(+) regulatory T cell suppression of dendritic cell function involves TGF-beta and IL-10. *Cancer Immunol Immunother* 2007; **56**: 48-59 [PMID: 16612596 DOI: 10.1007/s00262-006-0160-8]
- 104 **Bauer CA**, Kim EY, Marangoni F, Carrizosa E, Claudio NM, Mempel TR. Dynamic Treg interactions with intratumoral APCs promote local CTL dysfunction. *J Clin Invest* 2014; **124**: 2425-2440 [PMID: 24812664 DOI: 10.1172/JCI66375]
- 105 **Lai E**, Astara G, Ziranu P, Pretta A, Migliari M, Dubois M, Donisi C, Mariani S, Liscia N, Impera V, Persano M, Tolu S, Balconi F, Pinna G, Spanu D, Pireddu A, Saba G, Camera S, Musio F, Puzzone M, Pusceddu V, Madeddu C, Casadei Gardini A, Scartozzi M. Introducing immunotherapy for advanced hepatocellular carcinoma patients: Too early or too fast? *Crit Rev Oncol Hematol* 2021; **157**: 103167 [PMID: 33271389 DOI: 10.1016/j.critrevonc.2020.103167]
- 106 **Wing K**, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 2008; **322**: 271-275 [PMID: 18845758 DOI: 10.1126/science.1160062]
- 107 **De Martin E**, Michot JM, Rosmorduc O, Guettier C, Samuel D. Liver toxicity as a limiting factor to the increasing use of immune checkpoint inhibitors. *JHEP Rep* 2020; **2**: 100170 [PMID: 33205034 DOI: 10.1016/j.jhepr.2020.100170]
- 108 **McGonagle D**, Bragazzi NL, Amital H, Watad A. Mechanistic classification of immune checkpoint inhibitor toxicity as a pointer to minimal treatment strategies to further improve survival. *Autoimmun Rev* 2020; **19**: 102456 [PMID: 31838166 DOI: 10.1016/j.autrev.2019.102456]

- 109 **Michot JM**, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A, Massard C, Fuerea A, Ribrag V, Gazzah A, Armand JP, Amellal N, Angevin E, Noel N, Boutros C, Mateus C, Robert C, Soria JC, Marabelle A, Lambotte O. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer* 2016; **54**: 139-148 [PMID: 26765102 DOI: 10.1016/j.ejca.2015.11.016]
- 110 **Postow MA**, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *N Engl J Med* 2018; **378**: 158-168 [PMID: 29320654 DOI: 10.1056/NEJMra1703481]
- 111 **European Association for the Study of the Liver.** ; Clinical Practice Guideline Panel: Chair;; Panel members; EASL Governing Board representative:. EASL Clinical Practice Guidelines: Drug-induced liver injury. *J Hepatol* 2019; **70**: 1222-1261 [PMID: 30926241 DOI: 10.1016/j.jhep.2019.02.014]
- 112 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]
- 113 **Zhu AX**, Finn RS, Edeline J, Cattani S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]
- 114 **Duffy AG**, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, Davis JL, Hughes MS, Heller T, ElGindi M, Uppala A, Korangy F, Kleiner DE, Figg WD, Venzon D, Steinberg SM, Venkatesan AM, Krishnasamy V, Abi-Jaoudeh N, Levy E, Wood BJ, Gretten TF. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017; **66**: 545-551 [PMID: 27816492 DOI: 10.1016/j.jhep.2016.10.029]
- 115 **Ravi S**, Spencer K, Ruisi M, Ibrahim N, Luke JJ, Thompson JA, Shirai K, Lawson D, Bartell H, Kudchadkar R, Gunter NT, Mehnert JM, Lipson EJ. Ipilimumab administration for advanced melanoma in patients with pre-existing Hepatitis B or C infection: a multicenter, retrospective case series. *J Immunother Cancer* 2014; **2**: 33 [PMID: 25317333 DOI: 10.1186/s40425-014-0033-1]
- 116 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JJ, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Pérez-Gracia JL, Melero I, Prieto J. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013; **59**: 81-88 [PMID: 23466307 DOI: 10.1016/j.jhep.2013.02.022]
- 117 **Danlos FX**, Voisin AL, Dyevre V, Michot JM, Routier E, Taillade L, Champiat S, Aspeslagh S, Haroche J, Albiges L, Massard C, Girard N, Dalle S, Besse B, Laghouati S, Soria JC, Mateus C, Robert C, Lanoy E, Marabelle A, Lambotte O. Safety and efficacy of anti-programmed death 1 antibodies in patients with cancer and pre-existing autoimmune or inflammatory disease. *Eur J Cancer* 2018; **91**: 21-29 [PMID: 29331748 DOI: 10.1016/j.ejca.2017.12.008]
- 118 **Abdel-Wahab N**, Shah M, Lopez-Olivo MA, Suarez-Almazor ME. Use of Immune Checkpoint Inhibitors in the Treatment of Patients With Cancer and Preexisting Autoimmune Disease: A Systematic Review. *Ann Intern Med* 2018; **168**: 121-130 [PMID: 29297009 DOI: 10.7326/M17-2073]
- 119 **Tison A**, Quéré G, Misery L, Funck-Brentano E, Danlos FX, Routier E, Robert C, Loriot Y, Lambotte O, Bonniaud B, Scalbert C, Maanaoui S, Lesimple T, Martinez S, Marq M, Chouaid C, Dubos C, Brunet-Possenti F, Stavris C, Chiche L, Beneton N, Mansard S, Guisier F, Doubre H, Skowron F, Aubin F, Zehou O, Roge C, Lambert M, Pham-Ledard A, Beylot-Barry M, Veillon R, Kramkimel N, Giaccherio D, De Quatrebarbes J, Michel C, Auliac JB, Gonzales G, Decroisette C, Le Garff G, Carpiuc I, Vallerand H, Nowak E, Cornec D, Kostine M; Groupe de Cancérologie Cutanée; Groupe Français de Pneumo-Cancérologie, and Club Rhumatismes et Inflammations. Safety and Efficacy of Immune Checkpoint Inhibitors in Patients With Cancer and Preexisting Autoimmune Disease: A Nationwide, Multicenter Cohort Study. *Arthritis Rheumatol* 2019; **71**: 2100-2111 [PMID: 31379105 DOI: 10.1002/art.41068]
- 120 **De Martín E**, Michot JM, Papouin B, Champiat S, Mateus C, Lambotte O, Roche B, Antonini TM, Coilly A, Laghouati S, Robert C, Marabelle A, Guettier C, Samuel D. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. *J Hepatol* 2018; **68**: 1181-1190 [PMID: 29427729 DOI: 10.1016/j.jhep.2018.01.033]
- 121 **Day D**, Hansen AR. Immune-Related Adverse Events Associated with Immune Checkpoint Inhibitors. *BioDrugs* 2016; **30**: 571-584 [PMID: 27848165 DOI: 10.1007/s40259-016-0204-3]
- 122 **June CH**, Warshauer JT, Bluestone JA. Is autoimmunity the Achilles' heel of cancer immunotherapy? *Nat Med* 2017; **23**: 540-547 [PMID: 28475571 DOI: 10.1038/nm.4321]
- 123 **Sekai I**, Hagiwara S, Watanabe T, Kudo M. A case with hepatic immune-related adverse events caused by nivolumab exhibiting impaired accumulation of regulatory T cells. *Clin J Gastroenterol* 2021 [PMID: 33665689 DOI: 10.1007/s12328-020-01317-y]
- 124 **Alissafi T**, Banos A, Boon L, Sparwasser T, Ghigo A, Wing K, Vassilopoulos D, Boumpas D, Chavakis T, Cadwell K, Verginis P. Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. *J Clin Invest* 2017; **127**: 2789-2804 [PMID: 28581446 DOI: 10.1172/JCI92079]

- 125 **Eli Lilly and Company.** A Randomized Phase 2 Study of LY2157299 Versus LY2157299 - Sorafenib Combination Versus Sorafenib in Patients With Advanced Hepatocellular Carcinoma. [cited 10 February 2021]. In: ClinicalTrials.gov [Internet]. Indianapolis (IN): U.S. National Library of Medicine. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT02178358?term=Galunisertib&cond=Hepatocellular+Carcinoma&draw=2&rank=3> ClinicalTrials.gov Identifier: NCT00417417
- 126 **Giannelli G,** Mikulits W, Dooley S, Fabregat I, Moustakas A, ten Dijke P, Portincasa P, Winter P, Janssen R, Leporatti S, Herrera B, Sanchez A. The rationale for targeting TGF- $\beta$  in chronic liver diseases. *Eur J Clin Invest* 2016; **46**: 349-361 [PMID: 26823073 DOI: 10.1111/eci.12596]



Published by **Baishideng Publishing Group Inc**  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

**Help Desk:** <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

