Study Protocol

Organ-specific responses to atezolizumab plus bevacizumab in patients with advanced hepatocellular carcinoma: A multinational multicenter study

NCT Number:

NCT04862949

Version:

Version 1.3

Document Date:

April 27, 2023

1. Clinical Study Protocol

Study Title: Organ-specific responses to atezolizumab plus bevacizumab in patients with advanced hepatocellular carcinoma: A multinational multicenter study

Target Disease: Advanced hepatocellular carcinoma

Investigational Products:

Atezolizumab and Bevacizumab

Laboratory/Diagnostic Tests: Not applicable

Principal Investigator:

Hong Jae Chon, Division of Hematology and Oncology, CHA Bundang Medical Center, Korea

E-mail: minidoctor@cha.ac.kr Tel: +82-31-780-7590

2. Summary

Study Title:	Organ-specific responses to atezolizumab plus bevacizumab in patients with advanced hepatocellular carcinoma: A multinational multicenter study
Study Objective	The purpose of this study is to analyze organ-specific responses to the combination of atezolizumab and bevacizumab in patients with advanced hepatocellular carcinoma (HCC) and to evaluate their impact on survival outcomes. • **Primary Objective:** Organ-specific response rate
Principal Investigator	Hong Jae Chon, Division of Hematology and Oncology, CHA Bundang Medical Center
Participating Institutions	Ulsan University Hospital, Korea. The Chinese University of Hong Kong
Study Population	Patients with advanced hepatocellular carcinoma who received atezolizumab plus bevacizumab between May 2020 and June 2021
Study Design	Retrospective analysis using medical records
Evaluation Method	Organ-specific responses and survival outcomes of patients treated with atezolizumab plus bevacizumab were analyzed.
Statistical Analysis	For categorical variables, chi-square test or Fisher's exact test was used. Survival outcomes for each variable were estimated using the Kaplan–Meier method, and survival curves were compared with the log-rank test.

3. Objectives

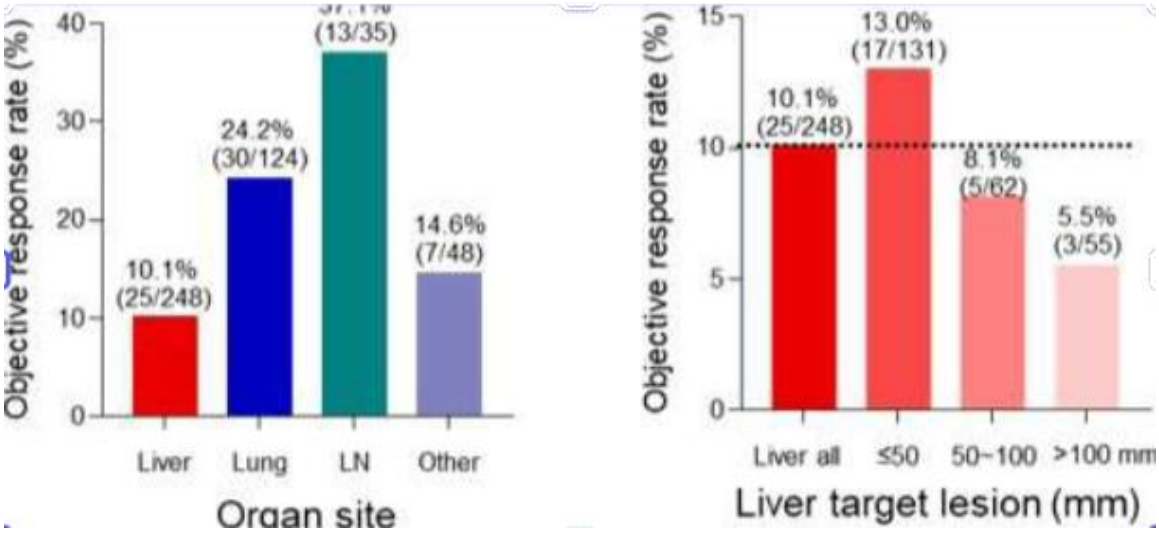
The purpose of this study is to identify the organ-specific responses of atezolizumab plus bevacizumab combination therapy in patients with advanced hepatocellular carcinoma (HCC), and to analyze the impact of these responses on survival outcomes.

Primary Objective: Organ-specific response rate
Secondary Objectives:
- Objective response rate (ORR) according to RECIST v1.1
- Progression-free survival (PFS)
- Overall survival (OS)

4. Background

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide and in Korea. Many patients are diagnosed at an advanced stage, where systemic therapy is the mainstay of treatment.{3,4}

Immune checkpoint inhibitors (ICIs) as monotherapy have shown responses in early-phase trials, but failed to significantly improve survival in phase 3 trials. Retrospective analyses suggest organ-specific response variability: intrahepatic lesions respond less favorably than extrahepatic metastases. VEGF overexpression plays a role in HCC progression. Anti-VEGF therapy can reduce immunosuppression, enhance T-cell infiltration, and improve ICI responses. Thus, the combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) has strong biological rationale and demonstrated clinical benefit in phase 3 studies.

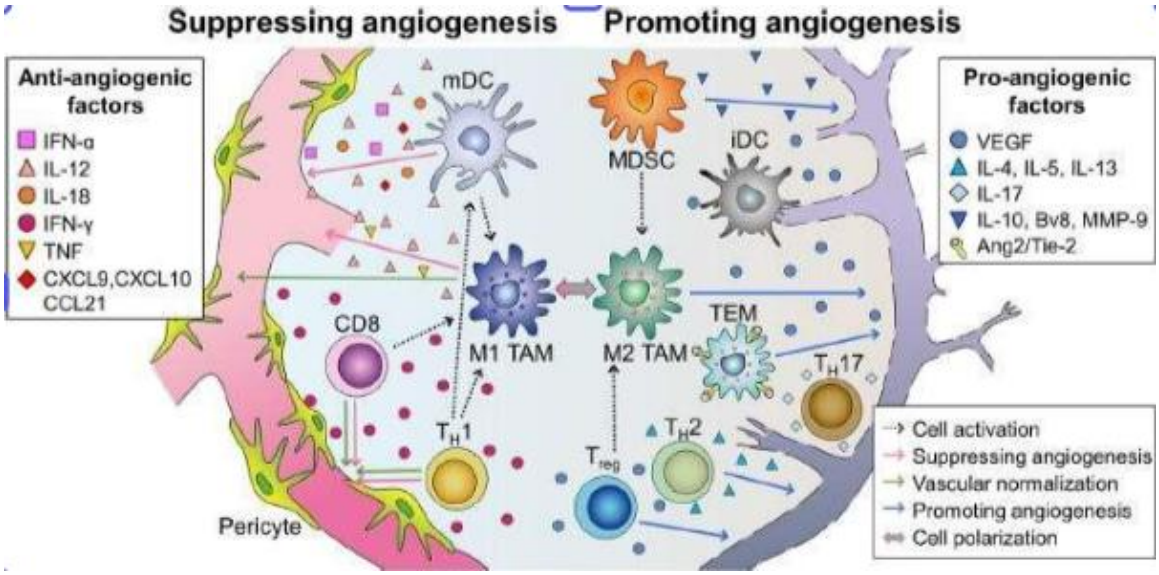


Organs	Liver	Lung	Lymph	Others
Organ-specific response	9%	25%	37%	15%

Kim HS, Cheon J, Chon HJ et al. Different organ-specific response to nivolumab to determine the survival

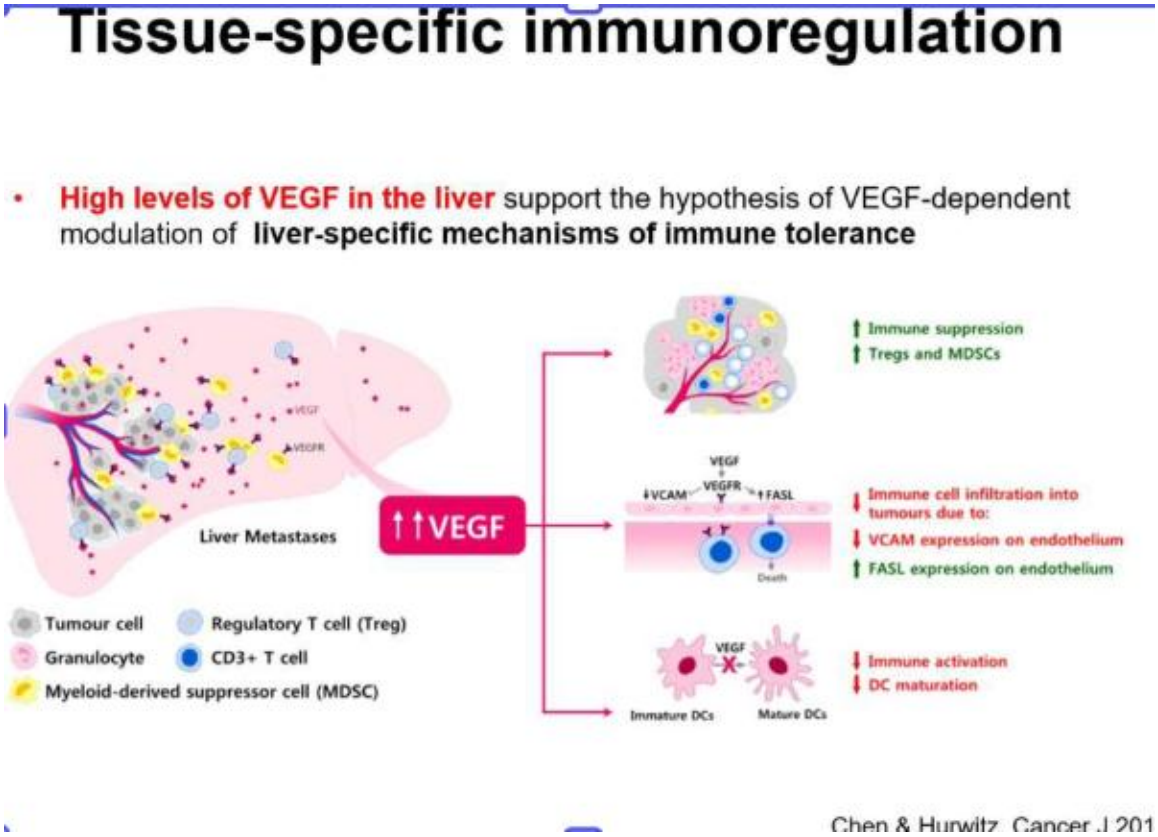
outcome of patients with advanced hepatocellular carcinoma (aHCC), ASCO 2020 Annual Meeting

Overexpression of vascular endothelial growth factor (VEGF) and activation of its downstream pathways have been associated with the development and progression of hepatocellular carcinoma (HCC) [10].



Chon HJ, Kim C et al. Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular-immune crosstalk to potentiate cancer immunity, Experimental & Molecular Medicine 2020

Anti-VEGF therapy can reduce VEGF-mediated immunosuppression within the tumor and its microenvironment, and by reversing VEGF-mediated immunosuppression, it can promote T-cell infiltration into tumors and thereby enhance responses to immune checkpoint inhibitor (ICI) therapy [11,12].



Based on preclinical data and results from a phase Ib clinical trial, a global, multicenter, open-label phase III clinical trial, **IMbrave150**, was conducted [13]. In the IMbrave150 trial, the combination of atezolizumab and bevacizumab demonstrated superiority over sorafenib in the first-line treatment of patients with advanced hepatocellular carcinoma (HCC) in terms of overall survival (OS) and progression-free survival (PFS) according to RECIST v1.1 criteria. Furthermore, the objective response rate (ORR) improved to 27.3% [14]. Considering that ICI monotherapy has shown ORRs of approximately 17–20%, the combination of atezolizumab and bevacizumab provided an enhanced response [5,6,7,15].

It is expected that combining atezolizumab and bevacizumab may overcome the immunosuppressive microenvironment of the liver and improve intrahepatic responses to immune checkpoint inhibitors (ICIs).

The mechanism of action of ICIs is explained by reactivation of inactivated T cells within the tumor microenvironment. Since the tumor microenvironment differs by organ, the therapeutic efficacy of ICIs may vary depending on the site of the tumor [16].

In retrospective analyses of patients treated with pembrolizumab for advanced melanoma and non–small cell lung cancer (NSCLC), those with liver metastases showed shorter PFS and lower ORRs compared to those without liver metastases (melanoma: 33.3% vs. 71.4%; NSCLC: 28.6% vs. 56.7%) [17]. Similar findings were reported in patients with metastatic triple-negative breast cancer, where pembrolizumab showed no response in patients with liver metastases [18]. Collectively, these results suggest that liver metastases, regardless of cancer type, exhibit lower responses to ICIs compared with metastases in other organs.

In addition, ICI therapy in advanced HCC has demonstrated heterogeneous organ-specific responses [8,9]. The low intrahepatic response rate to ICIs may be explained by the liver-specific immunosuppressive microenvironment [19]. Previous studies have shown that myeloid-derived suppressor cells (MDSCs) and regulatory T cells accumulate in the liver and suppress antitumor cytotoxic T cells and NK cells [20,21]. Moreover, liver-resident cells—including hepatic stellate cells, liver sinusoidal endothelial cells, and macrophages—induce resistance and dysfunction in cytotoxic T cells [22–25]. To overcome this immunosuppressive hepatic microenvironment, strategies such as combining ICIs with other agents to induce stronger antitumor immune responses or to modulate the immunosuppressive microenvironment are required.

Since the major cause of death in most patients with HCC is intrahepatic disease progression or hepatic failure due to underlying cirrhosis, the intrahepatic response rate to ICIs is a critical determinant of overall prognosis in advanced HCC [26].

Therefore, we hypothesize that combination therapy with atezolizumab and bevacizumab can increase organ-specific responses in patients with advanced hepatocellular carcinoma and thereby improve survival outcomes.

5. Subjects and Methods

Patients with advanced hepatocellular carcinoma who received combination therapy with atezolizumab and bevacizumab between May 2020 and June 2021 at CHA Bundang Medical Center, Ulsan University Hospital, and The Chinese University of Hong Kong will be analyzed for organ-specific response rates and survival outcomes.

[Measurement of Organ-Specific Response Rate]

In previous studies, the concept of "organ-specific response rate" was proposed to evaluate heterogeneous responses to immune checkpoint inhibitor therapy by organ [8].

The largest lesion representing each involved organ will be selected (up to 2 lesions per organ, with a maximum of 5 lesions per patient). Each lesion will be measured unidimensionally and evaluated according to RECIST v1.1 criteria:

- **Complete Response (CR):** Complete disappearance of the lesion or lymph node short axis < 1.0 cm
- **Partial Response (PR):** At least 30% decrease in size
- **Progressive Disease (PD):** At least 20% increase in size
- **Stable Disease (SD):** Neither CR, PR, nor PD

New lesions were not always indicative of disease progression but were added to the original target lesions to determine the total tumor burden.

Response evaluation will be performed by blinded, independent radiologists who will review and measure the images.

5.1 Inclusion Criteria

Patients will be eligible for inclusion if they meet all of the following criteria:

- Histologically or non-invasively confirmed hepatocellular carcinoma (HCC) according to the **American Association for the Study of Liver Diseases (AASLD)** criteria
- **ECOG performance status** of 0 or 1
- Patients who received combination therapy with **atezolizumab and bevacizumab** as **first-line systemic treatment** for unresectable HCC
- **Barcelona Clinic Liver Cancer (BCLC) stage B or C**
- **Child-Pugh class A** liver function
- At least one measurable lesion
- Adequate hematologic and organ function

5.2 Exclusion Criteria

Patients will be excluded from the study if they meet any of the following criteria:

- History of autoimmune disease
- Use of therapeutic-dose anticoagulants
    - Exception: Low-dose aspirin for cardiovascular protection is permitted

6. Rationale for Sample Size

As this is a multicenter retrospective study, there is no precise statistical basis for sample size calculation. It is estimated that approximately 124 patients per year are diagnosed with advanced hepatocellular carcinoma and treated with Tecentriq (atezolizumab) plus Avastin (bevacizumab) at the Chinese University of Hong Kong, Ulsan University Hospital, and CHA Bundang Medical Center. Among these, about 90 patients from CHA Bundang Medical Center are expected to be included in this study.

This study will include patients with measurable disease. Organ-specific response criteria using RECIST v1.1 will be applied to evaluate objective responses of tumors located in the liver, lungs, lymph nodes, and other intra-abdominal sites to atezolizumab plus bevacizumab combination therapy.

7. Data Collection Methods

Data will be retrospectively collected through the electronic medical record (EMR) system. Extracted data will be transmitted electronically to the principal investigator following standard procedures. After extraction, all data will be stored in anonymized form.

Access to the data will be restricted to the principal investigator and sub-investigators participating in the study. Imaging data from each hospital will be anonymized, extracted, and sent to independent radiologists for review and evaluation.

8. Statistical Analysis Methods

Comparisons of categorical variables will be performed using the chi-square test. Survival curves will be estimated using the Kaplan–Meier method. Statistical

comparisons between survival curves will be conducted using the log-rank test, with a significance level of 5%.

Multivariate analysis will be performed using the Cox proportional hazards regression model. All available data will be included in the analysis. In general, missing data will not be imputed and will be treated as "missing" in the statistical analysis.

9. Expected Study Period

The study is expected to take approximately two years from the date of IRB approval until April 30, 2023.

Planned Timeline

- First data extraction: **April 30, 2021**
- Final data extraction: **December 31, 2022**
- Final study report: **February 28, 2023**
- Manuscript submission: **April 30, 2023**

10. Study Design

This study is a retrospective study. After identifying patients who meet the inclusion and exclusion criteria, organ-specific responses and patient status will be evaluated through chart review, without any additional testing or administration of drugs.

11. Ethical Considerations

This is a retrospective study involving patients who have already received treatment at the study site, and the research will be conducted using anonymized data. As this is a retrospective study, obtaining informed consent is waived. The study will not involve any additional treatment or testing for the patients. Patient information will be minimized to avoid direct disclosure of personal data. Future research will be conducted in accordance with the Declaration of Helsinki and reviewed by the Institutional Review Board (IRB).

12. Data Quality Assurance and Data Management Plan

As this is a retrospective study, the use of direct patient identifiers will be minimized, and there will be no disclosure of personal information. Patient names, resident registration numbers, and hospital registration numbers will not be recorded. Measures will be taken to protect patient confidentiality.

Access to patient information will be restricted to the principal investigator and co-investigators. Patient information files will be encrypted and stored on computers accessible only to the principal investigator and co-investigators.

Collected data will be retained for five years. After the retention period, printed personal information will be destroyed using a shredder or incineration, and digital files will be permanently deleted using technical methods that prevent record recovery.

13. Data Items Collected in the Case Report Form (CRF)

The following information will be recorded in the case report form:

- Patient initials

- Study registration number
- Sex
- Age
- Date of birth
- Survival status
- Date of diagnosis
- Histological diagnosis (if available)
- Disease extent at the time of diagnosis
- Surgical history (yes/no) and date of surgery
- Clinical stage
- Pathological findings
- Recurrence status and date of recurrence
- Presence of recurrence or metastasis
- Response evaluation according to **RECIST v1.1**

13. Reference

1. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol. 2018 Oct;15(10):599-616.

2. Hong S, Won YJ, Park YR, Jung KW, Kong HJ, Lee ES. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2017. Cancer Res Treat. 2020 Apr;52(2):335-50.

3. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018 Jul;69(1):182-236.

4. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018 Mar 31;391(10127):1301-14.

5. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017 Jun 24;389(10088):2492-502.

6. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, et al. CheckMate 459: A randomized, multicenter phase III study of nivolumab (NIVO) vs sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). Annals of Oncology. 2019;30:v874-v75.

7. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, et al. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. J Clin Oncol. 2020 Jan 20;38(3):193-202.

8. Lu LC, Hsu C, Shao YY, Chao Y, Yen CJ, Shih IL, et al. Differential Organ-Specific Tumor Response to Immune Checkpoint Inhibitors in Hepatocellular Carcinoma. Liver Cancer. 2019 Nov;8(6):480-90.

9. Kim HS, Hong JY, Cheon J, Kim I, Kim CG, Kang B, et al. Different organ-specific response to nivolumab to determine the survival outcome of patients with advanced hepatocellular carcinoma (aHCC). Journal of Clinical Oncology. 020;38(15_suppl):4584-84.

10. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med. 2015 Feb 9;212(2):139-48.

11. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nat Med. 2014 Jun;20(6):607-15.

12. Hegde PS, Wallin JJ, Mancao C. Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. Semin Cancer Biol. 2018 Oct;52(Pt 2):117-24.

13. Lee M, Ryoo BY, Hsu CH, Numata K, Stein S, Verret W, et al. Randomised efficacy and safety results for atezolizumab (Atezo) + bevacizumab (Bev) in patients (pts) with previously untreated, unresectable hepatocellular carcinoma (HCC). Annals of Oncology. 2019;30:v875

14. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med. 2020 May 14;382(20):1894-905.

15. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a nonrandomised, open-label phase 2 trial. Lancet Oncol. 2018 Jul;19(7):940-52.

16. Pao W, Ooi CH, Birzele F, Ruefli-Brasse A, Cannarile MA, Reis B, et al. Tissue-Specific Immunoregulation: A Call for Better Understanding of the "Immunostat" in the Context of Cancer. Cancer Discov. 2018 Apr;8(4):395-402.

17. Tumeh PC, Hellmann MD, Hamid O, Tsai KK, Loo KL, Gubens MA, et al. Liver Metastasis and Treatment Outcome with Anti-PD-1 Monoclonal Antibody in Patients with Melanoma and NSCLC. Cancer Immunol Res. 2017 May;5(5):417-24.

18. Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. Ann Oncol. 2019 Mar 1;30(3):397-404.

19. Eggert T, Greten TF. Tumor regulation of the tissue environment in the liver. Pharmacology & therapeutics. 2017 May;173:47-57.

20. Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007 Jun 20;25(18):2586-93.

21. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Krüger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. Gastroenterology. 2008 Jul;135(1):234-43.

22. Berg M, Wingender G, Djandji D, Hegenbarth S, Momburg F, Hämmerling G, et al. Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells leads to tumor-specific CD8+ T cell tolerance. European journal of immunology. 2006 Nov;36(11):2960-70.

23. Li H, Wu K, Tao K, Chen L, Zheng Q, Lu X, et al. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. Hepatology (Baltimore, Md). 2012 Oct;56(4):1342-51.

24. Wu Y, Kuang DM, Pan WD, Wan YL, Lao XM, Wang D, et al. Monocyte/macrophage-elicited natural killer cell dysfunction in hepatocellular carcinoma is mediated by CD48/2B4 interactions. Hepatology (Baltimore, Md). 2013 Mar;57(3):1107-16.

25. Ji J, Eggert T, Budhu A, Forgues M, Takai A, Dang H, et al. Hepatic stellate cell and monocyte interaction contributes to poor prognosis in hepatocellular carcinoma. Hepatology (Baltimore, Md). 2015 Aug;62(2):481-95.

26. Uka K, Aikata H, Takaki S, Shirakawa H, Jeong SC, Yamashina K, et al. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. World J Gastroenterol. 2007 Jan 21;13(3):414-20.