Review

DOI: 10.5582/bst.2020.01039

The immunological characteristics of gallbladder carcinoma and advances in immunotherapy practices

Haihong Cheng[1], Di Zhou[2], Shouhua Wang[2], Jun Ding[3,*], Fei Ma[1,4,*]

[1] Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[2] Department of General Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[3] Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affiliated with the Shanghai University of T.C.M., Shanghai, China;
[4] Shanghai Institute for Pediatric Research, Shanghai, China.

SUMMARY Gallbladder carcinoma (GBC) is one of the most common malignant tumors in the biliary system, ranking sixth among gastrointestinal malignancies. In addition, the incidence of GBC has recently increased in China. GBC metastasizes early and invades adjacent organs such as the liver, making patients with GBC ineligible for radical surgery and giving them a poor prognosis. What is more, GBC is more inclined to develop chemo-resistance, which requires new strategies for clinical intervention. Cancer immunotherapy has made great advances over the past few years, with improved clinical efficacy against multiple malignancies, including GBC. This review summarizes the immunological characteristics of GBC as well as current advances in immunotherapies for GBC in order to provide new insights into future treatment and prevention of GBC.

Keywords gallbladder carcinoma, immunological characteristic, immunotherapy practices, vaccine, adoptive immunotherapy, cytokine

1. Introduction

Gallbladder carcinoma (GBC) is one of the most common primary malignancies of the biliary tract, with a higher incidence in women than in men. More than 90 percent of GBC are adenocarcinomas, which are moderately or poorly differentiated in most cases (*1*). The prognosis for GBC is extremely poor, with a median overall survival (OS) of around 4-7 months (*2*). At present, surgery, radiation, and systemic chemotherapy are the main treatments for GBC depending on the tumor stage and grade. The standard first-line chemotherapy regimen for advanced GBC, gemcitabine (Gem) and cisplatin (GC), has an objective response rate (ORR) of only around 30%. The median OS is only 11.7 months (*3*) and the 5-year overall survival rate is less than 5% after chemotherapy. Hence, exploring new treatment options is crucial to improving the survival of patients.

Unlike conventional therapies targeting tumor cells directly, cancer immunotherapy modulates the host's immune responses to induce sustained anti-tumor immunity and restrict tumor growth. Comprehensive strategies for cancer immunotherapy have been formulated depending on the immunological characteristics of the tumor itself and the host. In recent years, successful examples of cancer immunotherapy include the adoptive transfer of immune cells (*4,5*) and immune checkpoint blockades (*6,7*). In addition, tumor vaccines have also been developed with apparent efficacy in pre-clinical studies. Attempts at cancer immunotherapy for GBC are promising. This review summarizes current insights into the immunological characteristics of GBC and frontiers in immunotherapeutic approaches for GBC, which will facilitate the identification of new options to improve the clinical efficacy of therapies for GBC.

2. Immunological characteristics of GBC

The immune system plays a vital role in the development and progression of tumors. It can monitor the "non-self" mutant cells in the body and eliminate them specifically through cell immune mechanisms to maintain homeostasis. However, mutant cells may evade monitoring by the immune system through various mechanisms including low levels of expression of MHC molecules and tumor antigens, blocking of tumor antigens, and release of immunosuppressive factors. With these mechanisms, mutant cells can rapidly proliferate in an uncontrolled manner (*2*). In addition, the immune system can promote processes such as angiogenesis to promote tumor progression *via* the tumor microenvironment (*2*). Thus, immune therapy

should play a vital role in the treatment of a variety of cancers. Treating malignant tumors of the biliary system by regulating the immune system has become a hot topic of recent research (*8*). Both the innate immune system and the adaptive immune system warrant study.

Malignancies with different origins have the same features. Immune cell infiltration can be found in the tumor microenvironment. Differences in the pattern of infiltration affect the prognosis for a tumor (*2*). An increasing number of studies has indicated that substantial infiltration of macrophages, neutrophils, and regulatory T cells (Tregs) predicts a poor prognosis, while substantial infiltration of cytotoxic T lymphocytes (CTL) and mast cells indicates a better prognosis. Wang *et al*. established an immune index model based on the distribution of 5 types of infiltrating immune cells in GBC tissues, and they found that patients with a lower immune index ($Macrophage^{low}$ Neu^{low} $Treg^{low}$ CTL^{high} MC^{high}) had a better survival rate (*9*) .

2.1. The innate immune system

The innate immune system recognizes tumor antigens, induces and strengthens the adaptive immune system, and can kill tumor cells directly. In the tumor microenvironment, however, these effects are suppressed. Numerous studies have attempted to manipulate innate immunity to fight tumors (*10*). The cells involved in innate immunity primarily include mono-nuclear phagocytes, neutrophils, NK cells, NKT cells, γδT cells, and mast cells. Macrophages can be divided into two different types depending on their state of activation and function: M1 macrophages and M2 macrophages, or respectively classically activated macrophages and alternatively activated macrophages. M2 macrophages are associated with a poor prognosis in many primary tumors. Recent studies have indicated that CCL18 secreted by M2 macrophages promotes the migration and invasion of GBC cells *via* the PI3K/Akt pathway (*11*), which provides a new direction for research into targeted therapy for GBC. Neutrophils have been proven to be correlated with a lower survival rate for patients with head and neck malignancies or breast cancer (*2*). The neutrophil-lymphocyte ratio (NLR) is the most studied index in GBC. An increased NLR implies a poor prognosis (*12-15*). Du *et al*. found that the derived neutrophil-to-lymphocyte ratio (dNLR), lymphocyte-to-monocyte ratio (LMR), Fib-to-pAlb ratio (FPR), and CEA are closely related to the clinical prognosis for patients with metastatic GBC (mGBC). They are both independent risk factors that affect the prognosis for patients with mGBC. Among the aforementioned indices, dNLR has the highest predictive effect (*16*). Mast cells can inhibit tumor progression by releasing cytokines such as TNF or recruiting dendritic cells (DC), CD8+ T cells and NK cells. Bo *et al*. explored the relationship between tumor-infiltrating mast cells (TIMs) and the prognosis for patients with GBC treated with Gem. Gene enrichment analysis indicated that TIMs are related to the activation and recruitment of CD8+ T cells. Patients with advanced GBC and a high number of TIMs can significantly benefit from chemotherapy and have promising outcomes (*17*). γδ+T cells can recognize antigens not restricted by MHC. Recently, a study found that γδ+T cells are the main source of IL-17, which can promote tumor angiogenesis in patients with GBC. Blocking the production of IL17 *via* this pathway may be a new way to treat the disease (*18*) .

2.2. The adaptive immune system

The adaptive immune system mainly involves DC, CD4+ T lymphocytes, CD8+ T lymphocytes, and B lymphocytes. In the process of immune regulation, CD4+Treg cells inhibit the activation and proliferation of T cells; CD8+CTL and CD4+Treg cells are crucial cells in the anti-tumor immune response (*19*). T cell infiltration in human cancers determines the clinical outcomes of tumor to a certain extent. Antigen recognition in the tumor microenvironment plays a significant role in the function of T cells. Identifying therapeutic targets that could simultaneously improve T cell function is warranted.

Numerous studies on GBC also have focused on the adaptive immune system. Foxp3 is one of the vital transcription factors that control the development and function of CD4+ Treg cells. An immunohistochemical analysis of 80 patients with GBC found that CD8+ T cells were related to the improved survival time of patients with advanced GBC while Foxp 3+ T cells indicated a poorer prognosis (*20*). Oguro *et al*. recently discovered a co-inhibitory receptor, B and T lymphocyte attenuator (BTLA), that is structurally and functionally similar to cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death receptor 1(PD-1) and that is expressed on most lymphocytes. BTLA should be a novel therapeutic target by reversing the immune escape of tumors and enhancing anti-tumor immune function (*21*). In addition, a high platelet to lymphocyte ratio (PLR) is also independently associated with a poor prognosis in patients with GBC (*22*).

3. Cancer Immunotherapy for GBC

Thanks to the rapid advances in cancer immunology and immunotherapy, GBC immunotherapy has become a viable option in clinical trials and clinical practice. Current mainstream immunotherapies include therapeutic vaccines, adoptive immunotherapy, immune checkpoint inhibitors, and cytokines. Table 1 summarizes the completed clinical trials and Table 2 summarizes the ongoing clinical trials on immunotherapies for GBC. Due to the low incidence

Table 1. Clinical studies of immunotherapies for GBC

Immunotherapy	Treatment regimens	Phase	Targeted disease	OS (mo)	PFS (mo)	*Ref.*
Peptide-based vaccine (WT1)	Peptide vaccine+ gemcitabine	I	Pancreatic, GBC, ICC, ECC	9.3	–	(*8*)
CAR-T	CAR-T+ CTX+ nab-P	I	CC, GC	–	4	(*23*)
INF-α	5-FU+INF-α	–	BTC	11.9	6.2	(*24*)

BTC: biliary tract carcinoma.; CC: cholangiocarcinoma; CTX: cyclophosphamide; ECC: extrahepatic cholangiocarcinoma; GBC: gallbladder cancer; ICC: intrahepatic cholangiocarcinoma; INF-α: interferon-α; nab-P: nab-paclitaxel; OS: overall survival; PFS: progression-free survival.

Table 2. Ongoing clinical trials of immunotherapies for GBC

Treatment regimen	Phase	Estimated date of completion	Targeted disease	Identification number
Afatinib + Nivolumab	–	December 31, 2020	GBC	ChiCTR1800018149
Camrelizumab + Cap + radiotherapy	II	June 2024	BTC, ECC, GBC	NCT04333927
Atezolizumab + Cobimetinib	II	June 30, 2021	GBC, ICC, CC	NCT03201458
Bintrafusp alfa + Gem + Cis	II/III	July 24, 2023	BTC, CC, GBC	NCT04066491
Durvalumab + Gem/Cis	II	December 30, 2022	BTC, GBC, CC	NCT04308174
Avelumab + Peposertib + Radiotherapy	I/II	December 3, 2022	GBC, Solid Neoplasm, (and 26 more...)	NCT04068194
Tumor-infiltrating Lymphocytes	I/II	October 2022	Gastrointestinal Cancer, Pancreatic Cancer, GBC, (and 8 more...)	NCT04426669
Ipilimumab + Nivolumab	II	August 1, 2021	GBC, Acinar Cell Carcinoma, Adenoid Cystic Carcinoma, (and 91 more...)	NCT02834013
Pembrolizumab + Gem + Cis	III	August 31, 2023	BTC	NCT04003636
Manganese Chloride + nab-P + Gem + anti-PD-1 antibody	I/II	August 31, 2021	BTC	NCT04004234
AZD6738 + Durvalumab	II	March 31, 2022	BTC	NCT04298008
Durvalumab + Tremelimumab	II	December 2023	ICC	NCT04238637
Pembrolizumab	II	August 2021	BTC	NCT03110328

BTC: biliary tract carcinoma; CC: cholangiocarcinoma; CTX: cyclophosphamide; ECC: extrahepatic cholangiocarcinoma; GBC: gallbladder cancer; Gem: gemcitabine; ICC: intrahepatic cholangiocarcinoma; INF-α: interferon-α; nab-P: nab-paclitaxel OS: overall survival; PFS: progression-free survival.

of GBC, patients with that disease have mostly been included in clinical trials on BTC. The information about ongoing clinical trials was obtained from the Chinese Clinical Trial Registry and ClinicalTrials.gov.

3.1. Tumor vaccines

Current research on immunotherapy for GBC is mainly focused on peptide vaccines and DC vaccines (*25*). mRNA vaccines and DNA vaccines may also be promising areas of research in the future. Wilm's tumor 1 (WT1) and Mucin 1 (MUC1) are currently two antigens studied mostly in BTC. According to recent studies, both have been found to be associated with a poor prognosis in patients with cancer (*26*). A combination of Gem and WT1 vaccine was investigated in a Phase I study to treat unresectable GBC, cholangiocarcinoma, and pancreatic cancer (*8*). An increase in WT1-specific lymphocytes was observed. Moreover, the toxicity of the vaccine was negligible. In 2017, long-term remission was achieved in a patient with stage IV GBC who was treated with a combination of chemotherapy (Gem and titanium silicate-1) and an autologous formalin-fixed tumor vaccine containing autologous tumor fragments (*27*), suggesting the prospective clinical use of vaccines to treat GBC.

Traditional peptide vaccines have the disadvantage of low immunogenicity and MHC restriction. In contrast, DC vaccines can activate stronger immune responses by presenting specific antigens or tumor lysates. Since the first use of DCs loaded with melanoma-associated antigen (MAGE-1) to treat malignant melanoma *in vitro* in 1995, more than 400 clinical trials based on DC vaccines for the treatment of various malignant tumors have been conducted or completed worldwide (*28*). As early as 2005, researchers utilized tumor lysate antigens to produce 10 doses of autologous dendritic cell vaccines and successfully used them to treat a patient with stage III (T2, N1, M0) GBC; long-term remission (> 12 months) was achieved without metastasis (*29*). In a study in 2018, Rojas-Sepúlveda *et al*. selected GBC cell lines expressing tumor-associated antigens to prepare lysates (*25*). When subjected to heat shock, DCs become mature and capable of activating antigen-specific T cells. The study used allogeneic cancer cell lysates to prepare DC vaccines. This enhanced their efficacy, providing an alternative treatment for unresectable advanced GBC. The use of cell lysates

has many incomparable advantages. DCs can present a variety of antigens concurrently, and the damage-associated molecular patterns (DAMPs) produced by GBC cells under conditions such as heat shock can also promote the maturation and activation of DCs (*30*). The vaccine production process, such as the selection of DCs, optimization of DC maturation, and transportation should be optimized in order to maximize the efficacy of vaccines in the future (*31*). Zhang *et al*. screened six MHC I candidate tumor antigens in mouse lung cancer cell lines to prepare neoantigen adjuvant vaccines and neoantigen-sensitized DC vaccines, and they concluded that DC vaccines can produce a stronger immune response in mouse models of tumors (*28*).

Therapeutic mRNA vaccines can provide complete antigens, which is an advantage over peptide-based vaccines. Choosing a suitable ionizable lipid material as a carrier can promote the delivery of mRNA and result in stronger and more specific immune activation (*32*). Currently, mRNA vaccines are being investigated in a series of phase I and II clinical trials involving malignant melanoma, prostate cancer, glioblastoma, colorectal cancer, breast cancer, and non-small cell lung cancer (*33*). Although mRNA-based vaccines seem to offer promise and have advantages according to preclinical research, clinical trials are currently limited to phase I and phase II trials. Therefore, there is a long way to go before using those vaccines in the clinical treatment of GBC, though they are certainly worth exploring further.

A DNA vaccine refers to a plasmid designed to deliver genes encoding tumor antigens (TA) to stimulate or enhance the immune response to tumor cells expressing TA. A slew of completed clinical trials tested the efficacy of DNA vaccines against breast cancer, cervical cancer, pancreatic cancer, prostate cancer, multiple myeloma, and malignant melanoma. To date, DNA vaccines have not been investigated in GBC. One of their disadvantages, such vaccines might not be able to overcome the immune escape given the lack of relevant antigens in some tumor cells. This problem may be overcome through the combined use of antigens or selection of optimal antigens (*34*).

As precision medicine continues to develop, personalized tumor vaccines have also become a hot topic of research. Tumor neoantigens expressed as a result of mutations in tumor cell-related genes have high immunogenicity, and personalized tumor vaccines are likely to be highly efficacious (*35*). However, the heterogeneity of tumor cells hampers the selection of the mutation with the best immunogenicity. Moreover, manufacturing personalized vaccines is time-consuming, laborious, and costly (*36*).

3.2. Adoptive immunotherapy

Adoptive immunotherapy means the infusion of peripheral blood or tumor-infiltrating immune cells that have been modified and expanded *in vitro* in order to facilitate tumor suppression. As early as 1998, there was a case report about the combination of anticancer drugs and adoptive immunotherapy (infused TILs and CTLs obtained by co-cultivation of peripheral immune cells and autologous tumor cells) to treat advanced GBC, and that combination was efficacious to a certain extent (*37*). The patient's quality of life was improved remarkably and there was no biliary drainage due to the effects of immunochemotherapy. In another case in 2014, infusion of autologous NK cells and activated T cells (extracting peripheral blood NK cells and T cells to activate and expand those cells *in vitro*) sustained remission for more than 6 months in a patient with stage IV GBC who had not benefited from surgery or chemotherapy (*5*). A case in Japan in 2018 indicated that a combination of chemotherapy and adoptive immunotherapy (starting with cytokine-induced killer cells (CIK) and later changing to DC-induced killer cells (DIK)) achieved a long-term survival of almost 10 years in a patient (*4*). In this case, the only reported immunotherapy-related adverse event was a mild fever that did not noticeably affect the patient's quality of life. In a newly completed phase I clinical trial of 19 patients with EGFR-positive advanced biliary system malignancies (14 patients with cholangiocarcinoma and 5 with GBC), patients received autologous T cell therapy with chimeric EGFR antigen receptors. Among the 17 patients who were ultimately evaluable, 1 had complete remission, 10 had stable disease, and median progression-free survival was 4 months (2.5-22 months). Notwithstanding some reactions like mucosal/cutaneous toxicities and acute pulmonary edema, the T cell infusion was tolerated (*23*). A phase I/II clinical trial using TILs to treat GBC and other solid tumors will finish in 2022 (NCT04426669).

3.3. Immune checkpoint inhibitors

Immune checkpoint molecules (Checkpoints) play an inhibitory role in immune regulation. Currently, the most studied targets include PD-1/programed cell death ligand 1 (PD-L1) and CTLA-4. A recent study found that PD-L1 expressed on DCs interacts with PD-1 on T cells, resulting in inhibition of the anti-tumor response after DCs present tumor antigens to T cells. The down-regulation of PD-L1 in DCs and the silencing of PD-1 on T cells may lead to enhanced T cell activation of DCs, thereby producing effective anti-tumor T cell responses (*38*). Mayoux *et al*. posited a new mechanism: since PD-L1 can bind to PD-1 and B7.1 (CD80), a PD-L1 blockade can reduce PD-L1 binding to B7.1 on DCs; increasing the interaction of B7.1 and CD 28 can enhance the activation of T cells (*7*). The above studies all noted the significant role of DCs in anti-PD-L1 therapy, suggesting that the combination of DC vaccines and Checkpoints has a huge potential.

Given the remarkable efficacy of monoclonal antibodies (MAb) against the PD-1/PD-L1 pathway in non-small cell lung cancer, renal cell carcinoma, malignant melanoma, and urothelial carcinoma (*39*), the US Food and Drug Administration (FDA) has approved its use to treat a number of solid tumors. The expression of PD-L1 in 174 patients with GBC was measured using tissue microarray technology (*39*). PD-L1 expression was found in 23% of cancer tissues, suggesting the potential to use anti-PD-L1 therapy in GBC. Recently, a patient with recurrent and metastatic GBC with a high level of PD-L1 expression (≥ 50%) significantly benefited from radiotherapy combined with nivolumab with no adverse events (*6*). A point worth highlighting is that the FDA has recently approved durvalumab for the treatment of BTC. A clinical trial (NCT03110328) is currently underway to evaluate the feasibility and efficacy of pembrolizumab in the treatment of BTC.

CTLA-4 is a homolog of CD28 that sends an inhibitory signal to T cells. Combined therapy with PD-1 and CTLA-4 antibodies has been effectively used to treat multiple tumors (*40*). More clinical trials are investigating double checkpoint inhibitors for the treatment of GBC. A phase II study indicated that durvalumab and tremelimumab combined with a GP chemo-regimen resulted in an OS of 20.7 months in 121 patients with BTC, as was reported at ASCO this year. More trials using these two drugs or other immunotherapy combinations are recruiting subjects or preparing final results, as shown in Table 2. In addition to double checkpoint inhibition, clinical trials are also examining combinations with chemotherapy, target therapy or radiotherapy, including NCT04333927, NCT03201458, NCT04003636, NCT04003636, and NCT04066491 (Table 2).

The BTLA described above, which is widely expressed in human T cells, is a promising target as well. Antibodies that block such molecules can enhance human T cell responses as monotherapy or in combination with anti-PD-1 treatment (*41*).

3.4. Cytokines

IL-2 is a key factor in maintaining the activation of T cells, and its clinical use in metastatic malignant melanoma and metastatic renal cell carcinoma has been approved by the FDA (*34*). The synergistic use of IL-2 and PD-1/PD-L1 blockers has been used in clinical trials on non-small cell lung cancer, bladder cancer, and malignant melanoma (*42*). Studies have found that IFN-γ can inhibit the differentiation of monocytes into M2 macrophages and promote the transformation of M2 macrophages into M1 macrophages. Sun *et al*. subcutaneously inoculated BALB/C nude mice with a human GBC cell line (GBC-SD) to create an animal model. After intratumor injection of recombinant mouse IFN-γ, levels of vascular endothelial growth factor and the extent of angiogenesis in tumor tissues decreased significantly (*43*). IFN-α can activate anti-tumor immunity by promoting T cells, NK cells, and DCs while inhibiting the activity of Treg cells (*24,44*). In one study, about 23 patients with ICC and 2 patients with GBC received combined therapy with 5-fluorouracil and IFN-α (*24*). The overall response rate was 24% (6 of 25 patients had partial remission). Only one patient developed Grade 4 anemia and received a blood transfusion. However, no adverse reactions resulted in the discontinuation of treatment. Both IL-24 and IL-37 have pro-apoptotic or inhibitory effects on GBC cells, and they are also expected to become one of the approaches to the treatment of GBC (*45,46*).

4. Conclusion

Thus far, great efforts have been made to devise the therapeutic strategies for and to improve the efficacy of non-surgical treatments of GBC, including devising new therapeutic approaches such as targeted drugs and vaccines. The results of numerous clinical trials that have examined the efficacy of immunotherapy, as mentioned earlier, are particularly encouraging. Moreover, numerous clinical studies of treatments for GBC are underway. A point of paramount importance is to capitalize on combined therapies such as combined use of chemotherapy and immunotherapy or use of immunotherapies targeting different pathways. These approaches should have a synergistic effect with minimal toxicity.

Given the relatively low incidence of GBC, few patients with GBC are admitted to any hospital. Thus, large-scale multi-center clinical studies are crucial to achieving expected breakthroughs.

Funding:This work was supported by the National Natural Science Foundation of China (grant no. 81802337), Shanghai Jiao Tong University (grant no. YG2017MS74), and the Shanghai Health Committee (grant no. 2020404447).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Lazcano-Ponce EC, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistuba II, de Ruiz PA, Urista GA, Nervi F. Epidemiology and molecular pathology of gallbladder cancer. CA Cancer J Clin. 2001; 51:349-364.
2. Palucka AK, Coussens LM. The basis of oncoimmunology. Cell. 2016; 164:1233-1247.
3. Park K, Kim KP, Park S, Chang HM. Comparison of gemcitabine plus cisplatin versus capecitabine plus cisplatin as first-line chemotherapy for advanced biliary tract cancer. Asia Pac J Clin Oncol. 2017; 13:13-20.
4. Kawamoto M, Wada Y, Koya N, Takami Y, Saitsu H,

Ishizaki N, Tabata M, Onishi H, Nakamura M, Morisaki T. Long-term survival of a patient with recurrent gallbladder carcinoma, treated with chemotherapy, immunotherapy, and surgery: A case report. Surg Case Rep. 2018; 4:115.
5. Bhamare S, Prabhakar P, Dharmadhikari A, Dedeepiya VD, Terunuma H, Senthilkumar R, Srinivasan T, Reena HC, Preethy S, Abraham SJ. Autologous immune enhancement therapy in a case of gall bladder cancer stage IV after surgical resection and chemotherapy yielding a stable non-progressive disease. J Cancer Res Ther. 2014; 10:752-754.
6. Kong W, Wei J, Liu J, Qiu Y, Shi J, He J, Su M, Xiao M, Liu B. Significant benefit of nivolumab combining radiotherapy in metastatic gallbladder cancer patient with strong PD-L1 expression: A case report. Onco Targets Ther. 2019; 12:5389-5393.
7. Mayoux M, Roller A, Pulko V, *et al*. Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. Sci Transl Med. 2020; 12.
8. Marks EI, Yee NS. Immunotherapeutic approaches in biliary tract carcinoma: Current status and emerging strategies. World J Gastrointest Oncol. 2015; 7:338-346.
9. Wang J, Bo X, Wang C, *et al*. Low immune index correlates with favorable prognosis but with reduced benefit from chemotherapy in gallbladder cancer. Cancer Sci. 2020; 111:219-228.
10. Demaria O, Cornen S, Daëron M, Morel Y, Medzhitov R, Vivier E. Harnessing innate immunity in cancer therapy. Nature. 2019; 574:45-56.
11. Zhou Z, Peng Y, Wu X, Meng S, Yu W, Zhao J, Zhang H, Wang J, Li W. CCL18 secreted from M2 macrophages promotes migration and invasion *via* the PI3K/Akt pathway in gallbladder cancer. Cell Oncol (Dordr). 2019; 42:81-92.
12. Beal EW, Wei L, Ethun CG, *et al*. Elevated NLR in gallbladder cancer and cholangiocarcinoma - making bad cancers even worse: Results from the US Extrahepatic Biliary Malignancy Consortium. HPB (Oxford). 2016; 18:950-957.
13. Liu F, Hu HJ, Ma WJ, Yang Q, Wang JK, Li FY. Prognostic significance of neutrophil-lymphocyte ratio and carbohydrate antigen 19-9 in patients with gallbladder carcinoma. Medicine (Baltimore). 2019; 98:e14550.
14. Zhang L, Wang R, Chen W, Xu X, Dong S, Fan H, Liu C. Prognostic significance of neutrophil to lymphocyte ratio in patients with gallbladder carcinoma. HPB (Oxford). 2016; 18:600-607.
15. Zhang Y, Jiang C, Li J, Sun J, Qu X. Prognostic significance of preoperative neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in patients with gallbladder carcinoma. Clin Transl Oncol. 2015; 17:810-818.
16. Du JH, Lu J. Circulating CEA-dNLR score predicts clinical outcome of metastatic gallbladder cancer patient. J Clin Lab Anal. 2019; 33:e22684.
17. Bo X, Wang J, Wang C, *et al*. High infiltration of mast cells is associated with improved response to adjuvant chemotherapy in gallbladder cancer. Cancer Sci. 2020; 111:817-825.
18. Patil RS, Shah SU, Shrikhande SV, Goel M, Dikshit RP, Chiplunkar SV. IL17 producing gammadeltaT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. Int J Cancer. 2016; 139:869-881.
19. Lin J, Long J, Wan X, Chen J, Bai Y, Wang A, Yang X, Wu Y, Robson SC, Sang X, Zhao H. Classification of gallbladder cancer by assessment of CD8(+) TIL and PD-L1 expression. BMC Cancer. 2018; 18:766.
20. Fluxa P, Rojas-Sepulveda D, Gleisner MA, Tittarelli A, Villegas P, Tapia L, Rivera MT, Lopez MN, Catan F, Uribe M, Salazar-Onfray F. High CD8(+) and absence of Foxp3(+) T lymphocytes infiltration in gallbladder tumors correlate with prolonged patients survival. BMC Cancer. 2018; 18:243.
21. Oguro S, Ino Y, Shimada K, Hatanaka Y, Matsuno Y, Esaki M, Nara S, Kishi Y, Kosuge T, Hiraoka N. Clinical significance of tumor-infiltrating immune cells focusing on BTLA and Cbl-b in patients with gallbladder cancer. Cancer Sci. 2015; 106:1750-1760.
22. Pang Q, Zhang LQ, Wang RT, Bi JB, Zhang JY, Qu K, Liu SS, Song SD, Xu XS, Wang ZX, Liu C. Platelet to lymphocyte ratio as a novel prognostic tool for gallbladder carcinoma. World J Gastroenterol. 2015; 21:6675-6683.
23. Guo Y, Feng K, Liu Y, Wu Z, Dai H, Yang Q, Wang Y, Jia H, Han W. Phase I study of chimeric antigen receptor-modified T cells in patients with EGFR-positive advanced biliary tract cancers. Clin Cancer Res. 2018; 24:1277-1286.
24. Yashima Y, Sato S, Kawai T, Sugimoto T, Sato T, Kanda M, Obi S. Intraarterial 5-fluorouracil and interferon therapy is safe and effective for nonresectable biliary tract adenocarcinoma. Hepatol Int. 2015; 9:142-148.
25. Rojas-Sepulveda D, Tittarelli A, Gleisner MA, Avalos I, Pereda C, Gallegos I, Gonzalez FE, Lopez MN, Butte JM, Roa JC, Fluxa P, Salazar-Onfray F. Tumor lysate-based vaccines: On the road to immunotherapy for gallbladder cancer. Cancer Immunol Immunother. 2018; 67:1897-1910.
26. Zhao L, Zhong L, Xu S, Li B. Immunotherapeutic approaches in cancer treatment. Anticancer Agents Med Chem. 2019; 19:204-205.
27. Imaoka Y, Kuranishi F, Miyazaki T, Yasuda H, Ohno T. Long-lasting complete response status of advanced stage IV gall bladder cancer and colon cancer after combined treatment including autologous formalin-fixed tumor vaccine: Two case reports. World J Surg Oncol. 2017; 15:170.
28. Zhang R, Yuan F, Shu Y, Tian Y, Zhou B, Yi L, Zhang X, Ding Z, Xu H, Yang L. Personalized neoantigen-pulsed dendritic cell vaccines show superior immunogenicity to neoantigen-adjuvant vaccines in mouse tumor models. Cancer Immunol Immunother. 2020; 69:135-145.
29. Khan JA, Yaqin S. Successful immunological treatment of gallbladder cancer in India--Case report. J Zhejiang Univ Sci B. 2006; 7:719-724.
30. Nigro A, Montico B, Casolaro V, Dal Col J. A novel dendritic cell-based vaccination protocol to stimulate immunosurveillance of aggressive cancers. Methods Mol Biol. 2019; 1884:317-333.
31. Perez CR, De Palma M. Engineering dendritic cell vaccines to improve cancer immunotherapy. Nat Commun. 2019; 10:5408.
32. Miao L, Li L, Huang Y, Delcassian D, Chahal J, Han J, Shi Y, Sadtler K, Gao W, Lin J, Doloff JC, Langer R, Anderson DG. Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. Nat Biotechnol. 2019; 37:1174-1185.
33. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - A new era in vaccinology. Nat Rev Drug

Discov. 2018; 17:261-279.
34. Lopes A, Vandermeulen G, Preat V. Cancer DNA vaccines: Current preclinical and clinical developments and future perspectives. J Exp Clin Cancer Res. 2019; 38:146.
35. Vormehr M, Diken M, Tureci O, Sahin U, Kreiter S. Personalized neo-epitope vaccines for cancer treatment. Recent Results Cancer Res. 2020; 214:153-167.
36. Schlom J, Gulley JL. Vaccines as an integral component of cancer immunotherapy. JAMA. 2018; 320:2195-2196.
37. Murakami K, Tanimura H, Yamaue H, Mizobata S, Noguchi K, Nakamori M, Shimamoto T. Clinical effect of immunochemotherapy for a patient with advanced gallbladder cancer: Report of a case. Surg Today. 1998; 28:923-928.
38. Hassannia H, Ghasemi Chaleshtari M, Atyabi F, Nosouhian M, Masjedi A, Hojjat-Farsangi M, Namdar A, Azizi G, Mohammadi H, Ghalamfarsa G, Sabz G, Hasanzadeh S, Yousefi M, Jadidi-Niaragh F. Blockage of immune checkpoint molecules increases T-cell priming potential of dendritic cell vaccine. Immunology. 2020; 159:75-87.
39. Neyaz A, Husain N, Kumari S, Gupta S, Shukla S, Arshad S, Anand N, Chaturvedi A. Clinical relevance of PD-L1 expression in gallbladder cancer: A potential target for therapy. Histopathology. 2018; 73:622-633.
40. Zhang E, Gu J, Xu H. Prospects for chimeric antigen receptor-modified T cell therapy for solid tumors. Mol Cancer. 2018; 17:7.
41. Rosskopf S, Leitner J, Zlabinger GJ, Steinberger P. CTLA-4 antibody ipilimumab negatively affects $CD4^+$ T-cell responses *in vitro*. Cancer Immunol Immunother. 2019; 68:1359-1368.
42. Barroso-Sousa R, Ott PA. Transformation of old concepts for a new era of cancer immunotherapy: Cytokine therapy and cancer vaccines as combination partners of PD1/PD-L1 inhibitors. Curr Oncol Rep. 2018; 21:1.
43. Sun T, Yang Y, Luo X, Cheng Y, Zhang M, Wang K, Ge C. Inhibition of tumor angiogenesis by interferon-gamma by suppression of tumor-associated macrophage differentiation. Oncol Res. 2014; 21:227-235.
44. Ma H, Jin S, Yang W, Zhou G, Zhao M, Fang S, Zhang Z, Hu J. Interferon-alpha enhances the antitumour activity of EGFR-targeted therapies by upregulating RIG-I in head and neck squamous cell carcinoma. Br J Cancer. 2018; 118:509-521.
45. Jia J, Li S, Gong W, Ding J, Fang C, Quan Z. mda-7/IL-24 induces apoptosis in human GBC-SD gallbladder carcinoma cells *via* mitochondrial apoptotic pathway. Oncol Rep. 2011; 25:195-201.
46. Wu TJ, Xu B, Zhao GH, Luo J, Luo C. IL-37 suppresses migration and invasion of gallbladder cancer cells through inhibition of HIF-1alpha induced epithelial-mesenchymal transition. Eur Rev Med Pharmacol Sci. 2018; 22:8179-8185.

Received December 5, 2020; Revised January 21, 2021; Accepted January 29, 2021.

**Address correspondence to:*
Fei Ma, Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China.
E-mail: mafei@xinhuamed.com.cn

Jun Ding, Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affifiliated to Shanghai University of T.C.M., Shanghai,201203, China.
E-mail: doctording0916@126.com

Released online in J-STAGE as advance publication February 7, 2021.