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Changes in Anti-tumor Immune Response After Resection of Hepatocellular Carcinoma

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II. List of Abbreviations

%	Percentage
°C	Degree celsius
µg	Microgram
µl	Microliter
ml	Milliliter
aCTL	Activated cytotoxic T lymphocytes
AFP	Alpha-fetoprotein
ALT	Alanine transaminase
APC	Antigen-presenting cells
AST	Aspartate aminotransferase
aTh	Activated helper T cells
aTregs	Activated regulatory T cells
BCLC	Barcelona clinic liver cancer staging
Bregs	Regulatory B cells
CD	Cluster of differentiation
cmCTL	Central memory cytotoxic T lymphocytes
cmT cells	Central memory T cells
cmTh	Central memory helper T cells
CNI	Calcineurin inhibitors
CRC	Colorectal cancer
CRP	C-reactive protein
CS	Conventional surgery

cs-memory B cells	Class-switched memory B cells
CT	Computed tomography
ctDNA	Circulating DNA
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T lymphocyte-saaociated protein 4
DC	Dendritic cells
DFS	Disease-free survival
eCTL	Effector cytotoxic T lymphocytes
emCTL	Effector memory cytotoxic T lymphocytes
emT cells	Effector memory T cells
emTh	Effector memory helper T cells
eTh	Effector helper T cells
FACS	Florescence activating cell sorting
FCA	Flow cytometry analysis
FCM	Flow cytometry
FMO	Fluorescence minus one
G-MDSC	Granulocyte myeloid-derived suppressor cells
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
ICI	Immune checkpoint inhibitor
IFN-γ	Interferon-γ
Ig	Immunoglobulin

IL	Interleukin
INR	International normalized ratio
LAT	Local ablation treatment
LR	Liver resection
LT	Liver transplantation
maTregs	Memory activated regulatory T cells
MC	Milan criteria
MDSC	Myeloid-derived suppressor cells
MHC	Major histocompatibility complex
miRNAs	MicroRNAs
M-MDSC	Mononuclear myeloid-derived suppressor cells
MRI	Magnetic resonance imaging
mTregs	Memory regulatory T cells
MWA	Microwave ablation
nCTL	Naïve cytotoxic T lymphocytes
NK	Natural killer cells
NKT	Natural killer T cells
NLR	Neutrophil-to-lymphocyte ratio
ns-memory B cells	Non-class-switched memory B cells
nTh	Naïve helper T cells
nTregs	Naïve regulatory T cells
OS	Overall survival
PB	Peripheral blood

PBMC	Peripheral blood monocyte cells
PD-1	Programmed cell death-1
PD-L1	Programmed cell death-ligand 1
pH	Potential of hydrogen
PIVKA-II	Protein induced by vitamin K absence II
POD	Postoperative day
POM	Postoperative month
PreOP	Pre-operation
Pre B cells	Precursor B cells
Pro B cells	Progenitor B cells
RAS	Robot-assisted surgery
RCT	Randomized controlled trials
RFA	Radiofrequency ablation
RFS	Recurrence-free survival
TACE	Transarterial chemoembolization
DEB-TACE	Transarterial chemoembolization with drug-eluting beads
TAMs	Tumor-associated macrophages
Th	Helper T cells
Th1	Type 1 helper T cells
Th2	Type 2 helper T cells
Th17	Type 17 helper T cells
TNF	Tumor necrosis factor
Tregs	Regulatory T cells

UCSF	University of California at San Francisco
VATS	Video-assisted thoracoscopic surgery
VEGF	Vascular endothelial growth factor
VI	Vascular infiltration
WBC	White blood cell

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, with its incidence increasing year on year.[1] Hepatitis and liver cirrhosis remain the most significant pathogenic factors in the occurrence and development of liver cancer.[2] There are many treatment options depending on the stage of the tumor and the underlying liver disease.[3, 4] An analysis of the available research reveals that the occurrence, development, and prognosis of most tumors, including HCC patients, are closely related to the immune status of patients.[5, 6]

1.1. Epidemiology, Risk Factors, and Prevention of HCC

According to the latest report, liver cancer ranked sixth for incidence and third for mortality compared to other malignant tumors. Globally, in 2020, there were approximately 906,000 new cases of liver cancer (4.7% of all malignant tumors), with 830,000 deaths (8.3% of all malignant tumor deaths). Liver cancer occurs more frequently in males. The geographical distribution also plays a role, with more cases reported in economically struggling areas, such as West Africa, North Africa, East Asia, and Southeast Asia. For instance, the incidence of liver cancer in Mongolia is much higher than in any other country, primarily due to the high rates of hepatitis infection and alcoholism in Mongolia (Figure 1). The most common types of liver cancers are HCC (75–85% of all liver cancers) and intrahepatic cholangiocarcinoma (10–15% of all liver cancers). Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, aflatoxin-contaminated food, obesity, type 2 diabetes, smoking, and severe alcohol abuse are all major risk factors for the development of HCC. The HBV vaccine is included in most countries' infant immunization programs, effectively reducing the infection rate of HBV. Antiviral therapy in patients with chronic hepatitis (HBV and HCV)

can prevent progression to cirrhosis and HCC development.[7] While the use of pan-genotypic direct-acting antiviral medications can cure most HCV infections, there is currently no effective method to prevent HCV infection, such as a vaccine. Therefore, further research is required to reduce the HCV infection rate.[8-10] In addition, a host of epidemiological studies have shown that coffee intake can reduce the risk of HCC in patients with chronic liver disease, but there are currently no dosage recommendations.[11, 12]

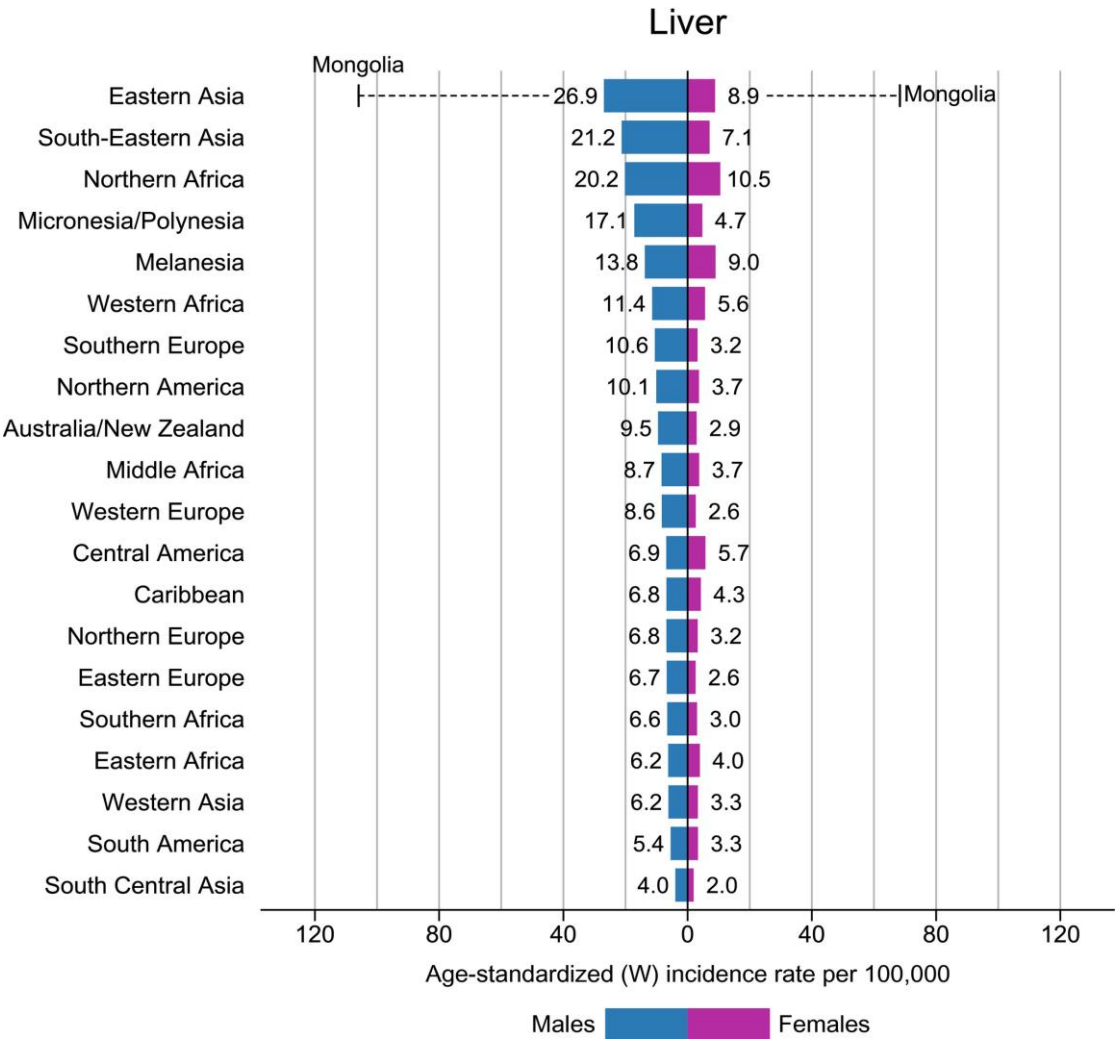

Figure 1. Estimated crude incidence rates of liver cancer, 2020.[10]

1.2. Early Detection of HCC

Regular surveillance of target populations at risk for a specific disease allows for early detection of patients, thereby improving the applicability and cost-effectiveness of treatment.

Ultrasound is the most widely used imaging test for surveillance of HCC based on its advantages of non-invasiveness, risk-free, low cost, easy acceptance, and early detection of complications of liver cirrhosis. When used as a surveillance test, it has high sensitivity (60–90%) and specificity (90%).[13] A meta-analysis showed that ultrasound can detect most HCC before clinical presentation with a sensitivity of 94%. However, detection of early HCC is less efficient, with a sensitivity of only 63%.[14] Furthermore, in the presence of very rough liver echo textures, the recognition of small tumors is also compromised. According to the 2018 EASL Clinical Practice Guidelines, abdominal ultrasonography every six months by an experienced professional is recommended for the following high-risk groups (moderate evidence; strong recommendation): 1. Patients with cirrhosis (Child-Pugh stage A and B); or patients with cirrhosis Child-Pugh stage C awaiting liver transplantation (LT) (low evidence; strong recommendation). 2. Non-cirrhotic HBV patients with intermediate or high HCC risk; or non-cirrhotic F3 patients (fibrosis score F3: severe liver scarring) (low evidence; weak recommendation).[7] In general, protein biomarkers are not recommended as early detection tools for HCC. They however can serve as indicators for tumor recurrence and progression or often in a scientific context as a prognostic marker.[15]

1.3. Relevant Biomarkers for HCC

Alpha-fetoprotein (AFP) is the most widely used biomarker in HCC. Persistently elevated AFP levels are a risk factor for the development of HCC and can be used to

help identify high-risk groups.[16] Using a threshold value of 20 ng/ml, the sensitivity of AFP for detecting HCC is 41–65%, with a specificity of 80–94%.[17] While sensitivity dropped to 22% with high specificity at a higher cut-off value of 200 ng/ml.[18] Reasons for poor AFP performance as a surveillance indicator include: fluctuations in AFP levels in patients with cirrhosis may reflect aggravation of the underlying liver disease, the outbreak of hepatitis virus infection, or the development of HCC. Besides, only approximately 20% of patients with small HCC present with elevated AFP.[19]

AFP-L3, a subtype of AFP, is recognized as an accurate HCC biomarker. Its specificity for detecting HCC has been reported to be 92.9%; however, its sensitivity appears to be low (48.3%). It could be combined with other biomarkers to improve the detection of small HCC.[20]

Protein induced by vitamin K absence II (PIVKA-II) has been identified as a potential serum biomarker for the detection of HCC. Its high diagnostic specificity can distinguish HCC from other non-malignant chronic liver diseases. PIVKA-II has similar diagnostic efficacy for both AFP-positive and negative HCC cases, making it a useful complement to AFP assessment.[21] However, its low detection sensitivity limits its clinical applications.[19]

MicroRNAs (miRNAs) are associated with HCC onset, proliferation, apoptosis, DNA repair, invasion, and metastasis and are abundant and easily detectable in plasma. A recent study confirmed the high diagnostic value of circulating miRNAs for HCC. The combined detection of miRNAs and AFP displays higher diagnostic accuracy than the detection of either alone, suggesting that this combination could be used as a comprehensive diagnostic algorithm for early HCC detection.[22, 23]

The advent of liquid biopsy may open up novel avenues for HCC diagnosis.[24] Unlike in traditional biopsies, circulating DNA (ctDNA) can be detected with liquid biopsies as

it is expressed extracellularly and is present in the plasma of cancer patients. Detection of early-stage HCC via liquid biopsy has demonstrated high sensitivity; however, its specificity is affected by other liver diseases. Furthermore, ctDNA expression levels are low in HCC patients, particularly during the early tumor stage. Using advanced molecular diagnostic techniques, ctDNA has been extensively investigated for possible applications in cancer diagnosis.[19]

Other biological markers have displayed potential diagnostic values for HCC, such as glypican-3,[25] golgi protein-73,[26] osteopontin,[27] dickkopf-1,[28] and long non-coding RNAs,[29] However, further study is required to confirm their clinical utility. The combined use of different methods and multiple markers may be a future trend for early HCC detection.

1.4. Diagnosis of HCC

HCC typically develops slowly, without obvious symptoms. This means it is often advanced when detected.[30] Approximately two-thirds of HCC patients have advanced, poorly treated HCC patients when first diagnosed, with a median survival of approximately 6–20 months after diagnosis.[31] Studies have confirmed that early detection of small tumors can lead to higher survival rates.[32] Due to HCC's high morbidity and mortality rates, early diagnosis is an effective way of improving patients' prognoses.

Unlike many other malignancies, HCC can be diagnosed based on imaging features alone.[33, 34] Magnetic resonance imaging (MRI) and computed tomography (CT) perform similarly in the diagnosis of HCC, providing a definitive diagnosis of lesions > 1 cm. For tumors ≥ 2 cm, the sensitivities of CT and MRI were 92% and 95%, respectively. However, for tumors < 2 cm, MRI performed better than CT, the

sensitivities were 62% and 48%, respectively.[35] MRI has high sensitivity and accuracy for the differential diagnosis of liver nodules. The use of liver-specific contrast agents in MRI improves sensitivity; however, such agents have limited efficacy in small HCCs. A prospective surveillance study found that MRI had a sensitivity of more than 85%.[36] MRI is used for intensive screening in high-risk or very high-risk populations after stratification and for diagnostic imaging of early-stage HCC patients. However, MRIs are costly and time-consuming. CT has a sensitivity of 66–73% in detecting early HCC patients, slightly higher than the sensitivity of ultrasound screening; however, the use of CT is limited by it being a medical source of radiation.[37]

Histopathological diagnosis is the gold standard for diagnosing HCC. Sensitivity for diagnosing HCC from liver biopsies of all tumor sizes has been reported to be 90%, and diagnostic specificity is almost 100% (except for differential diagnostic challenges).[38] However, due to the invasiveness current guidelines recommend non-invasive techniques in case of cirrhosis. In non-cirrhotic patients, a pathological confirmation should be undertaken. [7]

1.5. Staging Systems and Treatment Allocation for HCC

Although there are over a dozen HCC clinical classification methods worldwide. The Barcelona Clinic Liver Cancer (BCLC) classification system is the most commonly used clinical classification for HCC (Figure 2).[33, 39-41] The BCLC classification is more complex and comprehensive than the American Joint Committee on Cancer staging system as it includes information both on the extent of the tumor load (tumor size and number, extrahepatic metastases presence) and liver function (Child-Pugh score), as well as indicators of the patient's general health and ability to tolerate treatment (Eastern Cooperative Oncology Group score standard) to define the disease stage.[7, 42] The BCLC system is used to determine the prognosis and provide treatment

guidance for HCC patients at different clinical stages.[7] Since its inception the BCLC staging system has been widely adopted, however within the BCLC treatment allocation is debated in the literature. The following sections will provide a structured overview of the different BCLC stages and the recommended treatment strategies for each.

1.5.1. BCLC 0

Based on the BCLC staging criteria, very early-stage (BCLC stage 0) patients are fit, have a tumor smaller than 2 cm, and preserved liver function. Therefore, they can undergo either a partial liver resection (LR) or curative ablation. These patients often have a quick recovery and the chance for recurrence is comparably low since cirrhosis, which is regarded as precancerosis, is less pronounced. Data show that 80-90% of solitary HCC $<$ 2 cm in diameter survive 5 years after LR.[43]

1.5.2. BCLC A

BCLC A (early-stage) patients also have preserved liver function and a good performance state (Figure 2). However, these patients have a single large tumor (> 2 cm) or three nodules $\leqslant$ 3 cm. These patients should, if possible, be treated with either LR or LT. If the patients are a candidate for neither, ablation of the tumor/s should be attempted. The median 5-year survival rate of BCLC A patients who undergo LR, LT, or local ablation treatment (LAT) is between 50–70%.[7] The details of LR, LT, and LAT will be detailed in the treatment section of HCC below.

1.5.3. BCLC B

Intermediate-stage patients (BCLC stage B) suffer from multinodular (> 3 nodules) and unresectable tumors. In these patients, the liver function is mostly still preserved, and the physical state is acceptable. For BCLC stage B patients, the algorithm

recommends transarterial chemoembolization (TACE) as the primary treatment.[39, 44] BCLC stage B is the largest HCC subgroup. Its main feature is extensive heterogeneity in HCC patients. Therefore, the outcomes of TACE treatment are variable.[45] Clinical practice shows that the 2-year survival rate of patients with good liver function and small tumor size can be as high as 63%, which is better than that of patients with poor liver function and large tumor size.[46] Furthermore, studies have reported that the survival rate of patients with locally advanced HCC who undergo TACE treatment is significantly improved, with the relative risk of death reduced by more than 50%.[47, 48] However, as mentioned above some BCLC stage B HCC patients could also profit from more aggressive treatment with LT. Clinical analyses and studies could show that selected patients with diseases that exceed the confines of the Milan criteria (MC) can reach similar results as those within MC. Research has concentrated to find new surrogate markers for aggressive tumor biology beyond describing the mere size and number of tumor nodules. In Munich, researchers have opted to triangulate tumor aggressiveness by dynamically evaluating tumor growth within a 6-month waiting period, tumor response to LAT, and AFP levels (< 400 ng/ml). Based on the results of such research, it has been demonstrated that patients who fulfill all three requirements experience similar results as patients who meet the MC.[49] In general, possibilities to treat the heterogeneous group of BCLC stage B patients are still under debate and might change in the coming years, not least because also systemic therapies have made great advantages to treat patients with HCC.

1.5.4. BCLC C

For advanced-stage HCC (BCLC stage C), patients display a multilocular disease, with vascular invasion and/or extrahepatic spread in some cases and a reduced physical state. Patients with this stage tend to have a poor prognosis, with an expected median survival of 6-8 months, or 25% at one year.[50] Nonetheless, this outcome varied with

liver function status and other variables. With the introduction of Sorafenib survival for advanced cases has changed. It could be shown that sorafenib reached a survival benefit.[51] However novel therapeutics have surpassed the success reached with sorafenib and are now approved as first-line therapy. These are among others atezolizumab and bevacizumab.[52] There has been made great progress in the treatment of advanced HCC, which is described in more detail in the treatment section.

1.5.5. BCLC D

For patients with terminal-stage HCC (BCLC stage D), survival is limited due to decompensated liver cirrhosis and the lack of physical resilience of patients. Their median survival was 3-4 months or 11% at one year.[50] For these patients, the algorithm suggests that the best supportive care should be selected.[53]

In summary, LR, LT, and LAT are preferred for early-stage HCC, TACE is preferred for intermediate-stage HCC, and systemic therapy and/or supportive care are preferred for advanced-stage tumors.

1.6. Treatment of HCC

HCC treatment typically involves two major treatment types: surgical and non-surgical treatment. Surgical treatment is currently still the most effective treatment, such as LR and LT. Through further study of HCC's pathogenesis and the improvement of the technical aspects of treatments, multiple new therapies have emerged, including LAT, TACE, molecular targeted drug therapy, and immune checkpoint inhibitor (ICI).[39, 41, 54]

1.6.1. Liver Resection

Optimal surgical treatments are continuously researched. Ensuring the best result for

a patient, which in most cases is reached through LT, must be balanced against the shortage of organs available for transplantation and the need for immunosuppression following LT.[55] Therefore, in clinical practice, LR is the preferred treatment for HCC patients with small, isolated tumors and good liver function.[56] There are two basic principles of LR.[57-59] First is thoroughness, namely the complete removal of the tumor with no residual tumor left at the resection margin. 1) A solitary tumor with clear peripheral boundaries or pseudo-envelope formation, with < 30% of liver volume destroyed by tumor or > 30% of liver volume destroyed by tumor, but with significant compensatory enlargement of the tumor-free side of the liver (> 50% of entire liver volume.) 2) Multiple tumors with < 3 tumor nodules confined to one segment or lobe of the liver. 3) No invasion of the vasculature above the hepatic segment on imaging. 4) No extrahepatic metastatic tumors or only a single resectable metastatic tumor. The second is safety. A sufficient amount of functional liver tissue, namely tissue that has an adequate blood supply, blood, and bile reflux, should be preserved after surgery to compensate for postoperative liver function, reduce surgical mortality, and decrease surgical complications. It is generally believed that Child-Pugh grade A and indocyanine green clearance of more than 70% at 15 minutes is a necessary condition for surgical resection.[60] In cirrhosis patients, the remaining liver volume after resection should be more than 40% of the initial liver volume; in non-cirrhosis patients, it should be more than 30%.

Hepatectomy can be divided into two approaches, anatomic and non-anatomic LR. The liver is commonly divided into 8 functional segments.[61] Anatomic hepatectomy involves the removal of liver tissue from the corresponding hepatic segment, lobe, hemiport, or trilobe according to anatomic while pre-dissecting and blocking the incoming and/or outgoing hepatic blood flow to the liver. Anatomic hepatectomy enables a more complete resection of the lesion and reduces intraoperative bleeding and tumor recurrence. However, the procedure is difficult, and more non-tumoral liver

tissue is removed, which can affect the functional compensation of the residual liver.[62] Non-anatomic hepatectomy involves the resection of only the tumor and surrounding liver tissue, thereby preserving the maximum possible amount of non-tumor functional liver tissue. This procedure generally requires the resection margin to be at least 1 cm away from the tumor margin.[63] While non-anatomic resection is simple to perform, it is associated with an increased risk of tumor metastasis and recurrence.[64] Due to the complex hepatic vascular anatomy and abundant blood flow in the liver, significant intraoperative bleeding can lead to increased perioperative transfusion, which is associated with a high recurrence rate and low survival rate after LR.[65, 66] Therefore, the prognosis following a hepatectomy is closely related to adequate bleeding control during the operation. While LR can be achieved through conventional open hepatectomy, it can also be achieved via laparoscopic hepatectomy and robot-assisted laparoscopic hepatectomy.[67] There is no significant difference in prognosis between open and laparoscopic surgery. However, laparoscopic surgery may reduce postoperative complications and shorten the length of hospital stay.[68] With the application of robotic LR, its latent advantages in cases with higher difficulty levels are more obvious than that of laparoscopic surgery, which may have an impact on the treatment strategy of HCC.[69] However, HCC recurrence following LR remains a major problem, with the 5-year recurrence rate as high as 70%.[70] Recurrence can be divided into early (< 2 years) and late (> 2 years) recurrences. Early recurrences are caused by micro-metastases after resection, while late recurrences involve new tumors arising in a carcinogenic-prone microenvironment.[71] Available data suggest that further treatment of HCC recurrences through LR, LT, radiofrequency ablation (RFA), TACE, or systemic therapies display similar efficacies to when used to treat the primary HCC.[72] However, LT may be preferential in patients at a high risk of recurrence or after the recurrence develops.[73]

1.6.2. Liver Transplantation

LT is the most complete form of tumor resection. It removes precancerous tumors and reconstitutes liver function, thereby reducing the risk of liver failure after surgery.[74] It is suitable for patients with malignant liver tumors, benign end-stage liver disease, acute fulminant liver failure, or several congenital liver metabolic diseases.[75] There are several different techniques commonly used for whole postmortal LT. One is the conventional technique, which involves the resection and complete replacement of the post-hepatic inferior vena cava. The disadvantage of this technique is that the significant reduction in venous return in the anhepatic phase can result in hemodynamic changes.[76] Another is the piggyback technique, which preserves the posterior inferior vena cava during surgery.[77] The advantage of this technique is that it can maintain hemodynamic stability in the anhepatic stage, improving patient safety. The piggyback technique is the most widely used; however, hepatic vein stump anastomosis, which is involved in the technique, can easily lead to the distortion and obstruction of the outflow tract.

Split LT offers the possibility to increase the availability of this scarce resource. However, the use of split grafts in adults is rare.[78] Living donor LT developed under the same background as the split LT. Even though operative technologies have evolved living donor LT have been mainly limited to large centers with high case numbers and therefore acceptable results. The reason for this is the complexity of the technology and the morbidity and mortality risk for the donor. In addition, the recipient also faces the challenge of small size syndrome and biliary complications.

LT is the optimal choice for HCC patients who have impaired liver function (Child-Pugh grades B–C) and early-stage tumors or multiple small lesions (2–3 nodules, ≤ 3 cm each). This is because such patients have very low recurrence rates and high long-

term survival rates.[39, 40] While there are several selection criteria available to determine whether individual HCC patients meet the requirements for LT, the MC is the most commonly used. Based on the morphological characteristics of HCC prior to LT, the MC are the absence of vascular infiltration or extrahepatic metastasis, a diameter of less than 5 cm for a single tumor, or a diameter up to 3 cm each for up to 3 tumor nodules.[79, 80] Liver transplant recipients who met the MC often experience long-term survival. According to published results, the 5-year overall survival (OS) rate of liver transplant patients who meet the MC exceeds 70%, while the 5-year recurrence probability is approximately 8%.[80] A 2017 meta-analysis found that the 5-year OS rate of early HCC patients undergoing LT (66.67%) was higher than those undergoing LR (60.35%); however, there was no significant difference between 1-year and 3-year OS rates.[55]

Although patients who meet the MC experience high OS rates after LT, the accumulation of clinical studies and data on LT has uncovered several limitations of the MC. Firstly, It has been reported that some patients that exceed the MC can still obtain similar OS and recurrence-free survival (RFS) rates.[49, 81, 82] This shows that the MC is in part too strict, and excludes some patients that can profit from LT, making patients unable to receive this potentially curative treatment.[83] Secondly, the tumor size and number alone cannot accurately reflect the biological behavior of the tumor, and some HCC patients exceeding the MC can still benefit from LT after adjuvant treatment down to the MC. In addition, limited liver donor resources lead to long waiting times for transplantation and further tumor development even beyond MC, forcing some patients to drop off the transplantation waiting list.[40]

Based on these limitations, new liver transplant recipient selection criteria have emerged, such as the UCSF criteria, Up-to-7 criteria, Shanghai Fudan criteria, and Hangzhou criteria. These criteria ensure a similar OS rate to the MC while benefiting

more HCC patients.[84] However, most of these criteria are based on single-center retrospective analysis and are primarily focused on the expansion of tumor size and number. Therefore, using specific tumor markers as criteria, which are more reflective of tumor biology than tumor size or number, should be considered. However, tumor markers currently lack sufficient sensitivity and specificity. Therefore, exploring new tumor markers in multicenter, large sample studies and using them in combination with tumor size and number will help to establish more appropriate selection criteria for LT recipients.

Patients with HCC who exceed MC can obtain surgical opportunities through local downstaging treatment, which provides three goals for HCC candidates for LT. First, it can reduce the tumor burden within the MC in patients who initially exceed the MC (step-down therapy). Second, downstaging prevents patients who meet MC from dropping off the waiting list due to prolonged waiting time leading to tumor progression (bridging therapy). In addition, it can also reflect tumor biological behavior and sensitivity to neoadjuvant therapy, to have a better guiding significance for the prognosis of patients.[85] Effective therapies include TACE, transarterial radioembolization, and LAT.[86] A systematic review found that the success rate of HCC downstaging was greater than 40%, while the HCC recurrence rate following LT was 16%.[87] However, there are still limitations. First, the recurrence rate in downstaging patients is higher compared to those who meet the MC without downstaging. Second, no clear evidence to guide optimal treatment plans currently exists. Third, some tumors are not sensitive to the treatment, resulting in further progression while patients are waiting for transplantation. Therefore, data on downstaging before LT is eagerly awaited, to safely expand the recipient population and improve results after LT.

Recurrence is also dependent on the immunosuppression used. Compared to other organs, the liver is unique in being a preferential immune organ and is the organ most

likely to induce immune tolerance. However, graft rejection is a key factor that impacts the long-term survival of the transplanted liver and patient following LT.[88] The occurrence of immune rejection following LT is reduced via the routine use of immunosuppressive drugs. Calcineurin inhibitors (CNI) are the most important drugs for maintaining immunosuppression after LT. Cyclosporine A and tacrolimus are two CNIs that maintain immunosuppression by inhibiting the activity of helper T cells (Th) and B cells.[89, 90] Rapamycin (also known as Sirolimus) is a mammalian target of rapamycin（mTOR）inhibitors used for immune maintenance after LT,[91] and the main mechanism is to inhibit the proliferation of T and B cells.[92] Glucocorticoids were the first non-specific drugs used for immunosuppression and remain commonly used today. Glucocorticoids, such as hydrocortisone, have strong inhibitory effects on mono-macrophages, neutrophils, T cells, and B cells. Glucocorticoids are often used in combination with other drugs for basic treatment and are occasionally the first-line treatment of choice for immune induction and acute rejection.[93] However, the application of immunosuppressive agents can lead to the disruption of the immune surveillance system, resulting in the inability of the body to effectively remove tumor cells from the peripheral circulating blood. This, in turn, can allow these tumor cells to colonize and multiply in intrahepatic or extrahepatic organs or tissues, leading to tumor recurrence or metastasis.[94] The recurrence rate of HCC patients after LT is less than 20%.[95] Several studies have shown that high exposure to CNI after LT is an independent risk factor for postoperative tumor recurrence.[96, 97] Unlike CNI, rapamycin has been shown to decrease tumor proliferation in an experimental setting. In clinical use, however, there was no effect on RFS beyond 5 years after LT. Therefore, this anti-proliferative effect may only be relevant in certain patient subgroups.[98] Furthermore, there is a direct correlation between the long-term use of immunosuppressive drugs and post-transplant complications such as infection, malignant tumors, and renal failure.[75] Therefore, it is necessary to maintain a minimum level of

immunosuppression to prevent rejection and reduce the risk of recurrence while reducing the risk of developing post-transplant complications.[96] Other factors associated with post-transplant recurrence should also be recognized, such as tumor biological behavior, AFP levels, and transplantation waiting time, as they can be used to refine selection criteria for transplantation candidates and attempt to reduce recurrence.[99]

1.6.3. Local Ablation and External Radiation

RFA is the standard of care for patients with BCLC grade 0 and A tumors who are not appropriate for surgery, due to the safe margin of intact tumor necrosis that can be obtained in most cases.[7] Furthermore, RFA causes less surgical trauma than LR and can obtain better short-term postoperative results. However, RFA can cause heat damage and a heat sink effect. Therefore, it is not suitable for the treatment of HCC patients close to large blood vessels or other important organs.[100] Randomized controlled trials (RCT) have shown that, for suitable patients, RFA and LR have similar survival rates.[101] The guidelines set by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommend RFA as the first-line treatment for a single tumor less than 2 cm in size. The main predictor of treatment failure is tumor size, so RFA is recommended for small lesions.[102] Compared to RFA, microwave ablation (MWA) has a larger ablation zone. Three meta-analyses comparing percutaneous MWA and RFA showed that the efficacy of the two techniques is similar.[103-105] While MWA is a proposed treatment for early liver cancer, evidence regarding its efficacy is lacking.

A number of studies have reported the efficacy and tolerability of external beam radiotherapy techniques in different stages of HCC,[106] but there is still a lack of evidence to consider radiotherapy as an effective and validated option, and large

prospective studies are still needed.

1.6.4. Transarterial Therapies

TACE is considered the most widely used primary therapy for intermediate stage (stage B) unresectable HCC. [7] The basic principle is to inject cytotoxic agents into the hepatic artery that supplies nutrients to the tumor and embolize the nutrient artery, resulting in ischemic necrosis of the tumor. Contraindications are major portal vein branches or macrovascular invasions of the main portal vein.[48]

A recent systematic review of conventional TACE reported an objective response rate of 52.5% and an OS of 70.3%, 40.4%, and 32.4% at 1, 3, and 5 years, respectively, and the median OS was 19.4 months. Common adverse events included pain, blood/bone marrow toxicity, pyrexia, abnormal liver enzymes, and vomiting. The overall mortality rate was 0.6%, and the most common cause of death was related to acute hepatic insufficiency.[107]

TACE with drug-eluting beads (DEB-TACE) slowly releases cytotoxic agents and increases ischemia intensity and duration compared to conventional TACE.[108] A study comparing TACE with drug-eluting beads and conventional TACE showed that DEB-TACE has some advantages in terms of toxicity and radiation tumor response.[109] In contrast, another retrospective study showed that biliary tract injury, intrahepatic cholangiomas, and liver injury were higher after DEB-TACE than with conventional TACE, especially in patients with advanced cirrhosis.[110] In the literature, it is still debated which technology offers the greatest advantages for the diverse group of patients that can receive a TACE.

1.6.5. Systemic Therapies

Traditional chemotherapeutic drugs, such as cisplatin, and 5-fluorouracil against HCC show poor specificity, insignificant therapeutic effects, and toxic side effects on organs, especially the liver. In recent years, research on the application of molecularly targeted drugs has demonstrated promising results.[111] Sorafenib was the first first-line oral targeted therapy approved for unresectable HCC in 2007. Sorafenib has dual anti-tumor effects: it directly inhibits tumor cell proliferation by blocking cell signaling pathways mediated by RAF/MEK/ERK and indirectly inhibits tumor cell growth by blocking tumor angiogenesis via the inhibition of vascular endothelial growth factor (VEGF) receptors and PDGF receptors.[112-114] Results from a multicenter phase III RCT showed that median OS was improved by approximately 3 months in the sorafenib-treated group compared to the placebo group (10.7 vs. 7.9 months).[51] In a multinational phase III RCT in the Asia-Pacific region, some improvement in median OS was observed in the sorafenib-treated group compared to the placebo group (6.5 vs. 4.2 months).[115] The results of these two studies indicate that sorafenib could be used as the standard first-line treatment for advanced HCC patients. However, individual differences in the efficacy of sorafenib have been reported in clinical applications. Furthermore, tumors may easily develop resistance to the drug, although the mechanism of this resistance is unclear.[116] Therefore, there is a need to develop alternative drugs to address this drug resistance challenge. Lenvatinib is a selective, multi-target tyrosine kinase (including VEGFR1, 2, and 3, FGFR1, 2, 3, and 4, PDGFR α and β, cKIT, and RET) small molecule inhibitor.[117-119] The inhibition of FGFR4 is considered to be the core mechanism of lenvatinib's anti-tumor effect.[119] In vitro experiments have found that lenvatinib can dually inhibit the VEGF and FGF pathways and inhibit the proliferation signals of VEGFR and FGFR.[120] The results of a phase III multicenter REFLECT trial found that lenvatinib was not inferior to sorafenib in terms of OS but was superior in all secondary endpoints (objective response rate,

progression-free survival, and time to progression). In addition, the adverse effects associated with lenvatinib are typically controllable. Therefore, in 2018, several countries (including Germany) approved the use of lenvatinib as a first-line systemic treatment alternative to sorafenib for unresectable HCC patients.[117, 121, 122] Tumor growth and progression are associated with a suppressed immune system. Tumor cells can activate different immune checkpoint pathways that have immunosuppressive functions.[123] In HCC, the programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) are the relevant immune checkpoints.[124] The development of ICI is a milestone in the field of immuno-oncology, as they inhibit checkpoint-mediated signaling to reactivate tumor-specific T cells, exerting anti-tumor effects.[125] Nivolumab demonstrated a tumor response rate of approximately 20% in the Checkmate 040 study. In some cases, even a complete remission could be achieved.[126] Besides, pembrolizumab has achieved similar results as nivolumab (KEYNOTE-224).[127] In 2020, several ICIs were approved for the treatment of HCC in Germany. Results from the phase III IMBrave study demonstrated that atezolizumab (anti-PD-L1 monoclonal antibody) combined with bevacizumab (humanized anti-VEGF monoclonal antibody) showed better overall- and progression-free survival than sorafenib. For this reason, atezolizumab combined with bevacizumab are approved for first-line therapy in patients with unresectable HCC.[128, 129] In fact in clinical practice this therapy has emerged as a cornerstone of the treatment of unresectable HCC. Additionally, regorafenib, cabozantinib, ramucirumab, and nivolumab have the potential to be used as second-line treatment options and may improve OS rates in some patients.[117]

1.6.6. Palliative and Best Supportive Care

Patients with end-stage HCC (BCLC stage D) have a poor prognosis with a life expectancy of approximately 3-4 months.[130] There is no tumor-specific treatment at

this stage, only symptomatic and supportive treatment.[131] Pain is the most common symptom. For mild pain, acetaminophen is the drug of choice.[132] For moderate to severe pain, opioids are the drug of choice. However, cirrhosis may affect drug metabolism, and patients are at increased risk for hepatic encephalopathy.[133] Palliative radiotherapy is indicated when pain is caused by definite bone metastases, or when osteolytic bone metastases are considered to be at high risk for spontaneous fracture.[134] Furthermore, nutritional interventions and psychological support should not be ignored or underestimated.[131, 135]

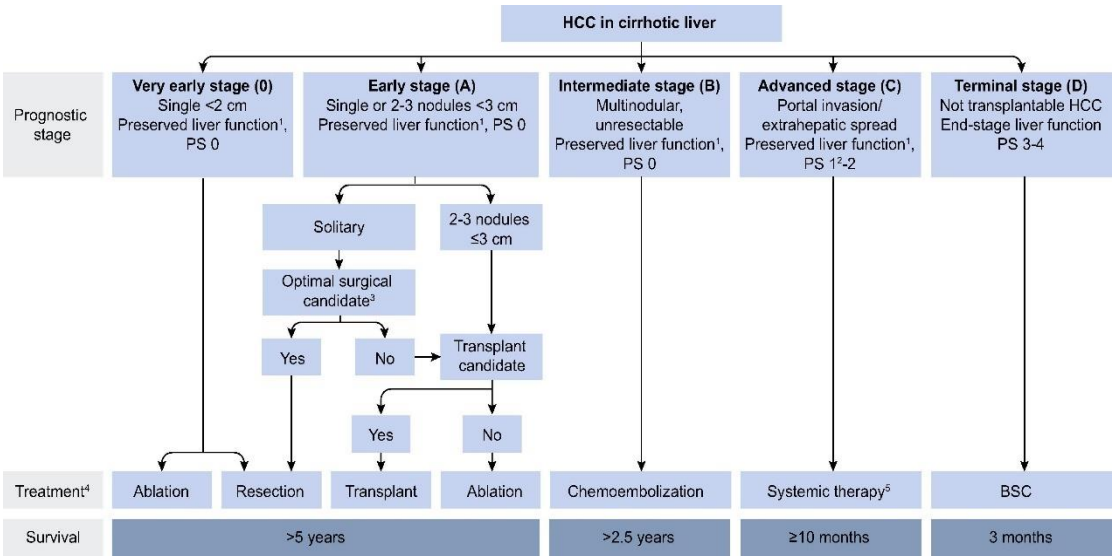

Figure 2. BCLC staging system and treatment strategy.[7]

1.7. Predictors for HCC After Surgery

Multiple markers can predict recurrence and survival after HCC resection, including the main aspects shown in Table 1.

Table 1. Predictors for HCC following surgery.

Patient-specific variables	Tumor-specific variables
Age	Number of tumors
Gender	Tumor nodule
Hepatitis	Vascular infiltration
Cirrhosis	Tumor microenvironment
Albumin level	Degree of tumor differentiation
AFP level	Tumor capsule
Alkaline phosphatase level	
Perioperative blood transfusion	
Operation time	

The impact of age on recurrence or survival following HCC surgery has been variably reported. Lam et al. claimed that age does not correlate with the long-term patient prognosis.[136] Conversely, Kim et al. reported that younger patients tend to have poorer prognoses as they tend to have more advanced-stage tumors.[137] Similarly, Chen et al. reported that younger patients (< 40 years old) had worse OS and 1-year survival rates following resection compared to older patients. The driving factor behind this difference may be the larger tumor sizes seen in younger patients, even if they have a better-preserved liver function.[138] The incidence and recurrence rates of liver cancer in

females are far lower than in males. Additionally, the prognosis is also better for females. Zhang et al. found that HCC has a higher incidence and worse disease-free survival (DFS) rates among males, which may be related to male hormone levels, as androgens may promote tumors, while estrogen may suppress them.[139] However, Park et al. reported that gender was not linked to OS or DFS rates.[140]

Some studies have demonstrated that active hepatitis is related to recurrence; patients with low quantitative levels of HBV display higher tumor-free survival rates.[141-143] Effective anti-HBV treatment can significantly reduce the recurrence rate and ameliorate prognoses for LR or LT.[144]

Cirrhosis is a common chronic liver disease that can evolve into HCC as the disease progresses. Additionally, it can affect the long-term survival rate of patients following HCC resection.[145] Studies have shown that patients with liver cirrhosis have significantly lower long-term survival rates than those without cirrhosis. In a multivariate analysis, cirrhosis was the only risk factor that affected OS rates after HCC resection.[146] Similarly, Portolani et al. reported that the most important risk factor for late recurrence after surgical HCC resection was cirrhosis due to the associated chronic liver damage.[147] Typically, the liver function of liver fibrosis patients is worse than those with normal livers, which may cause reduced blood coagulation, hypoalbuminemia, and decreased liver reserve function, which, in turn, can lead to an increase in perioperative complication incidence and a reduction in postoperative survival.[139]

Tumor size is negatively correlated with long-term survival. Compared to patients with small HCC patients, the survival rate of patients with larger HCC is very low after resection.[148] Chen et al. found that tumors smaller than 5 cm typically had good overall prognoses, but tumors smaller than 3 cm had better 5-year OS and DFS rate

predictions.[149] However, some researchers have pointed out that well-selected, large HCCs can still achieve good long-term survival rates after resection.[148] Two separate studies reported that the number of tumor nodules is an important evaluation index for HCC prognosis.[150, 151] In a univariate analysis, Park et al. reported that both OS and DFS rates were worse when multiple tumors were present (≥ 2 tumor nodules).[140] These results however are under dispute since in multivariate analyses the predictiveness of tumor number and size is less relevant than thought before.

The degree of tumor differentiation is an independent risk factor that significantly affects long-term survival after surgery. The lower the degree of differentiation, the higher the malignancy and invasiveness of HCC, and the worse the prognosis of the postoperative patient, so the degree of tumor differentiation can effectively predict the risk of postoperative recurrence.[66] HCC with good capsules is usually less likely to recur after surgery compared with no capsules, mainly since it usually does not invade the main vascular structure and surgical removal of the tumors can be relatively efficiently performed, resulting in a significant reduction in postoperative recurrence rate.[66, 151]

Immune cells present in tumor tissues play important roles in tumor immune microenvironments. Increasingly, studies claim that tumor-infiltrating immune cells are important for predicting prognoses.[152] Some results have confirmed that tumor-infiltrating leukocytes, particularly lymphocytes, can help predict HCC patient prognosis following transplantation or resection.[82, 153] Furthermore, Schoenberg et al. found that perivascular-infiltrating lymphocytes cluster of differentiation 3 (CD3) and CD8 are independent effective predictors of OS and DFS rates in postoperative HCC patients.[154]

Vascular infiltration (VI), including macrovascular and microvascular invasion, usually

occurs in advanced tumor stages and is closely related to the progression and prognosis of HCC.[155] Roayaie et al. reported that the presence of more than 5 VIs in the vascular wall can increase the recurrence rate of post-resection HCC, while a distance of less than 1 cm between VIs and tumors can predict poor postoperative survival results.[156] Park et al. found that HCC patients where the portal vein was invaded had lower OS and DFS rates compared to a control group, indicating that portal vein infiltration influences the prognosis of HCC patients.[140] Also, Bertuzzo et al. found that VI is closely related to prognosis post-LT.[157]

Tumor markers, including glycoproteins, embryonic antigens, and special proteins, can be found in blood, body fluids, or tissues. AFP is a commonly used auxiliary indicator for HCC diagnosis. Multiple studies have reported the prognostic significance of AFP for HCC. Liu et al. found that AFP levels of > 400 ng/mL impact prognosis after HCC resection.[158] Besides, univariate analysis results from Yeh et al. show that high levels of alkaline phosphatase may reduce OS rates after LR.[159] Preoperative hypoalbuminemia levels have also been found to be related to postoperative recurrence.[66, 160]

Operation time length and perioperative blood transfusion are also related to postoperative recurrence. The surgery itself is a trauma and will result in postoperative immunosuppression. Therefore, the shorter the operation time, the smaller the impact on the survival of patients. Blood transfusion increases the likelihood of thrombosis, which, in turn, promotes local colonization by cancer cells. In HCC, portal vein infiltration is usually accompanied by micrometastases, which facilitate cancer spread and the formation of further micro-metastases under the dual effects of surgical trauma and blood transfusion.[139, 161]

In summary, several factors can predict the recurrence and survival of patients

following HCC operations. A lack of immune response or immunosuppression is responsible for recurrence after resection or transplantation. Therefore, an effective strategy to prevent recurrence may involve the modification of the remaining immune response. However, no studies to date have reported the status of residual immune responses in HCC patients after resection. So it is necessary to have a comprehensive understanding of the tumor immune system after HCC surgery to determine the postoperative immunological characteristics of HCC, including the number and distribution of immune cells.

1.8. Immunology in the Context of HCC

The human immune system is comprised of innate and adaptive immune systems. In this section, these systems and their respective immune responses in HCC patients will be described.

1.8.1. Innate Immune System

Neutrophils are the primary cells involved in the immune defense response.[162, 163] Neutrophils also affect the initiation and regulation of adaptive immunity.[164, 165] Some reports indicate that neutrophils, like dendritic cells (DC), can present antigens to T and B cells.[166, 167] Granot et al. demonstrated that tumor-associated neutrophils exert cytotoxic effects on tumor cells and inhibit tumor metastasis.[168] However, other research has confirmed that tumor-associated neutrophils can induce tumor cell migration and invasion and promote tumor angiogenesis, thus promoting tumor development and deterioration.[169, 170]

Peng et al. suggested that poor OS and DFS rates in patients with small HCC are associated with elevated pre-operative neutrophil to lymphocyte ratios (NLR).[171] Li et al. found that the presence of neutrophil infiltration in tumors was also related to poor

outcomes following LR.[172] However, Schoenberg et al. did not find an effect of intra-tumoral neutrophil infiltration on HCC patient prognosis.[154]

Monocytes are a type of white blood cell (WBC) that can be circulated into tissues via the action of chemokines. They can be further differentiated into macrophages and DC. Monocytes are phagocytic, can present antigens, and can produce cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-12.[173] Previous research indicates that monocytes can also stimulate and regulate the immune system.[174] HCC patients following resection are related to high preoperative lymphocyte-to-monocyte ratios, which could be used as an independent prognostic factor.[175] Most peripheral monocytes enter human tissues and evolve into corresponding phagocytic cells, displaying phagocytic, immunoregulator, and antigen-presenting roles.[176] The liver contains the largest number of macrophages out of all body organs.[177] Previous studies have shown that monocytes near tumors can further differentiate into tumor-associated macrophages (TAMs). TAMs can accelerate tumor angiogenesis and enhance invasion and metastasis.[174, 178] The results from two meta-analysis studies indicate that a higher number of TAMs in the tumor microenvironment is correlated with decreased OS and DFS rates in HCC patients.[179, 180] In contrast, Atanasov et al. suggest that TAMs are correlated with enhanced postoperative survival of HCC patients.[181]

Mature DC are the strongest and most effective antigen-presenting cells (APC). They can ingest, degrade, process, and present antigens to naive T lymphocytes to initiative the adaptive immune response, serving as an important bridge between adaptive and innate immunity.[182] DC can also promote the generation of Th, cytotoxic T lymphocytes (CTL), and natural killer (NK) cells to exert their respective immune effects.[183, 184] Kakumu et al. reported that peripheral blood (PB) DC function is significantly weakened in HCC patients.[185]

Myeloid-derived suppressor cells (MDSC) are primarily comprised of two subtypes: mononuclear MDSC (M-MDSC) and granulocyte MDSC (G-MDSC, also known as polymorphonuclear MDSC).[186, 187] MDSC have powerful immunosuppressive functions, including the ability to suppress effector T cells, NK cells, DC, and macrophages, induce expansion and recruitment of regulatory T cells (Tregs), produce immunosuppressive cytokines, and promote tumor angiogenesis and cell spread.[188-190] One publication displayed that the circulating MDSC level of HCC patients was higher than in healthy people.[191] Another study reported that an increase in preoperative circulating MDSC level in HCC patients undergoing radical resection was associated with early recurrence and could therefore be used to predict patient prognosis.[192] Besides this, several experimental pieces of evidence show that high levels of MDSC infiltration in tumor lesions are associated with a poor prognosis.[193, 194]

NK cells primarily develop in the bone marrow. They are effector lymphocytes that possess a strong killing ability. Their killing mechanism involves the release of perforin and granzyme to destroy target cells;[195] however, they can also promote targeted cell death via TNF. NK cells play an important role in tumor monitoring. Compared to PB, the human liver has a richer lymphocyte content.[196] The tumor environment can increase the expression of transforming growth factor β, and increase the number of Tregs and MDSC, thereby destroying the immune effect of NK cells.[197] Cai et al. pointed out that the number of NK cells in the PB and tissue of HCC was reduced, meaning that their killing effect was also significantly reduced.[198] This reduction is related to postoperative recurrence and poor patient prognosis.[199] Natural killer T (NKT) cells co-express T cell and NK cell receptors.[200] When stimulated by an antigen, NKT cells produce chemokines, such as IL-2, 4, 5, 13, TNF-α, and interferon-γ (IFN-γ), which, in turn, work to regulate other immune cells.[200-203] NKT cells are enriched in the liver. While some reports suggest that they are related to chronic hepatitis, their

exact effect on HCC has not yet been uncovered.[204, 205]

1.8.2. Adaptive Immune System

T cells are the primary cells involved in the adaptive immune response. According to their functions and surface markers, they can be divided into multiple subtypes: Th, CTL, and Tregs.[206] Following antigen stimulation, Th can produce a variety of cytokines that promote the participation of related immune cells in the cellular immune response. Th can also drive B cells to produce antibodies for humoral immune responses. Via the action of specific transcription factors, Th can be further differentiated into type 1 helper T (Th1) cells, type 2 helper T (Th2) cells, and type 17 helper T (Th17) cells.[207] Th1 secrete the cytokines IL-1β, 2,12, IFN-γ, and TNF-α. The presence of Th1 is correlated with a good prognosis in HCC patients. Th2 cells produce IL-4, 5, 6, 9, 10, and 13. The main effect of Th2 cells is to assist B cell activation, and the cytokines secreted by Th2 can promote B cell proliferation and differentiation, and antibody production. It also can induce allergic reactions and participate in anti-parasitic infections.[208] Chen et al. reported that a decrease in the ratio of Th1/Th2 cytokines in the tumor microenvironment is closely related to the recurrence and metastasis of HCC.[209] Th17 produces cytokines IL-17, 21, and 22. Several reports suggest that Th17 may have an immunosuppressive function for HCC, accelerating their progression.[210, 211] Yan et al. reported that the increased ratio of Th17 to Th1 in PB is related to tumor progression and can be used as an index to evaluate the prognosis of HCC.[212] Possessing an immunosuppressive function, Tregs play an important role in maintaining the body's autoimmune tolerance and immune homeostasis.[213] Tregs inhibit T cell activity and secrete cytostatic factors.[214] Higher levels of circulating Tregs in the blood are associated with poorer HCC prognoses.[215] In tumor tissues, high-density tumor-infiltrating Tregs cells are associated with poorer HCC prognoses.[216] Tregs can inhibit the antitumor activity of NK cells.[217] CTL can kill

tumor cells by releasing cytotoxins (such as perforin and granzyme).[218] T cells can be divided into the naive stage, effector stage, and memory stage according to whether they have been stimulated by the antigen and the different stages of differentiation after being activated by the antigen.[219] Naive T cells are activated after their first contact with an antigen, which they recognize with the help of an APC. Most naïve T cells differentiate into effector T cells, which possess a killing function, and most die after the antigen is eliminated. However, a few differentiated into memory T cells with immune memory function. When memory T cells encounter the same antigen, they can be rapidly activated and differentiated into effector T cells and re-mediate the immune response.[219, 220] Under different effector functions and homing behaviors, memory T cells can be differentiated into central memory T cells (cmT cells) or effector memory T cells (emT cells).[221] emT cells produce cytokines IFN-γ and IL-4, migrate to the infection area and quickly display their effector function upon re-contact with the antigen. cmT cells secrete IL-2 and easily be transformed into effector cells following antigen stimulation.[221, 222]

B lymphocytes play a role in humoral immune response mediation by secreting antibodies to be recognized. B cells can also secrete cytokines and can present antigens to naive T cells.[223] B cells originate in the bone marrow and can differentiate into multipotent progenitor cells: common lymphoid progenitor cells, progenitor B (pro B) cells, precursor B (pre B) cells, immature B cells, Transitional B cells, and mature B cells.[224] Under antigen stimulation, some naive B cells differentiate into plasma cells that produce immunoglobulin (Ig) M. The remaining naive B cells develop into germinal center B cells, memory B cells, and plasmablasts,[225] which then develop into plasma cells capable of secreting IgM, IgG, and IgA.[226] The surface marker of memory B cells is CD27.[227] According to whether IgD is expressed or not, memory B cells can be divided into non-class-switched memory B cells (ns-memory B cells) that produce IgM or class-switched memory B cells (cs-memory B cells) that produce IgM, IgA, and

IgG.[228, 229] Regulatory B cells (Bregs) are a B-cell subtype that have negative immunoregulatory functions.[230] Bregs can inhibit the function of DC, macrophages, Th1, Th2, and Th17 through a variety of mechanisms.[231] Based on the results of co-cultures of Bregs and HCC cancer cells, Ding et al. find that Bregs can inhibit the apoptosis of HCC cancer cells.[232] In addition to anti-tumor effects, tumor-infiltrating B lymphocytes can promote tumor development under the influence of certain factors.[233]

1.9. Related Research

In this section, I explore related research that investigated perioperative changes in immune cell distribution after resection of liver tumors. For our initial search of the PubMed database, the following search terms ("Immune System" [Mesh]) AND (“Liver Neoplasms" [Mesh]) AND ("Perioperative Period" [Mesh]) were used. The last time point for the search was May 2018. We used prospectively defined inclusion and exclusion criteria to focus the literature for this review:

- **Literature inclusion criteria:** (1) Research type: clinical research; (2) Research object: human; (3) Research content: circulating immune cells; (4) Literature languages: English. (5) Perioperatively follow-up time-frame: from preoperational to 1 year after surgery.

- **Literature exclusion criteria:** (1) Published before 2000 or not in English; (2) Clinical trials, studies of therapy, etc; (3) Case reports, meta-analyses, or reviews; (4) Animal research; (5) Non-liver tumor; (6) Only preoperative data or only postoperative data; (7) Research on genes, non-immunological proteins, etc; (8) No surgery performed.

The retrieval strategy was first to browse the titles and abstracts of the publications, and select the articles that meet the inclusion criteria. The full-texts were downloaded

if available. The content extracted was the author's name, publication period, tumor origin, sample size, surgical method, specimen source, measurement method, immune cell type and corresponding marker, follow-up measurement time point, and immune cell change trend. References of retrieved full-texts were additionally scanned for relevant publications to reduce omissions.

To unify the results, we divided postoperative time-points into the following three time-frames: within 7 days after surgery, between 7 days after surgery up until 3 months after surgery, and from 3 months after surgery to 1 year after the operation.

Following the initial systematic search strategy, 47 relevant publications were identified. According to the exclusion criteria, only 2 publications studying HCC were eligible. Moreover, the types of cells that were studied were few, the observation and follow-up time after surgery was short, and could not be compared and analyzed. Based on this, we expanded the search scope to entire digestive system tumors. The search items were as follows: (“Immune System” [Mesh]) AND (“Digestive System Neoplasms” [Mesh]) AND (“Perioperative Period” [Mesh]). The rest of the screening requirements were the same as above (except that the exclusion criteria were adjusted from "non-liver tumor" to "non-digestive system tumors"), and 458 related publications were finally identified (Figure 3). Based on the exclusion criteria, 443 articles were excluded. Three additional publications were identified by scanning the references of the included publications. One paper was excluded as it did not contain full text.[234] Finally, 17 publications satisfied the predefined inclusion criteria and were included in this study. The research selection flowchart is depicted in Figure 3.

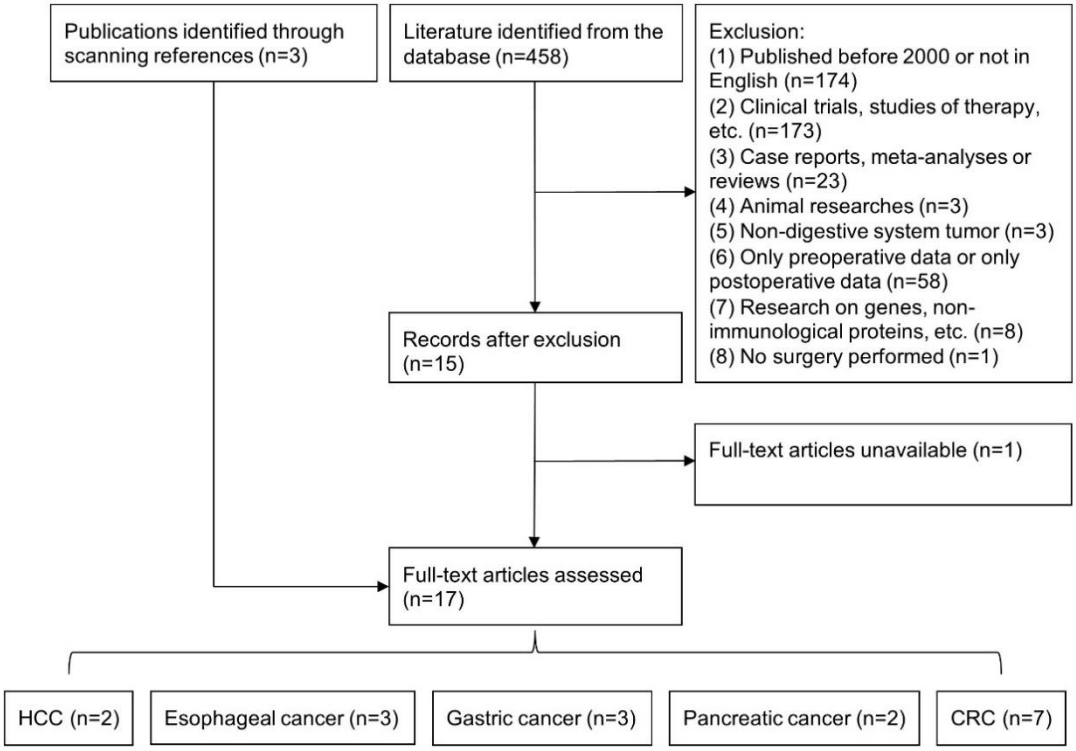

Figure 3. Flowchart of research selection.

The 17 studies (n = 1897 patients) examined changes in immune cells in digestive system tumors perioperatively and were included in this review. As can be seen in Figure 4A, the majority of the studies (n = 14; 82.35%) were conducted place in China and Japan.[171, 235-247] The remaining three studies were conducted in Europe (n = 3; 17.65%).[248-250]

The most common digestive system tumor type of included studies was colorectal cancer (CRC; n = 7; 41.18%).[236, 238, 239, 241, 245, 248, 249] Three reports on both gastric cancer and esophageal cancer were included (17.65% each).[235, 237, 240, 244, 246, 250] Samples from pancreatic tumor patients and HCC patients were measured in two separate studies (11.76% each; Figure 4B).[171, 242, 243, 247]

As shown in Figure 4C, 10 (58.82%) studies measured immune cells in fresh PB

samples.[235, 237-239, 241, 243, 245, 246, 249, 250] Four (23.53%) studies measured peripheral blood monocyte cells (PBMC) isolated from PB.[236, 240, 244, 247] Three studies did not describe the source of samples in detail (17.65%).[171, 242, 248]

Flow cytometric analysis (FCA) was used in more than half of the studies (n = 10; 58.82%).[236-240, 243, 244, 246, 247, 249] PB cell count analyses (as performed in a routine laboratory) were used in the remaining studies (n = 7; 41.18%; Figure 4D).[171, 235, 241, 242, 245, 248, 250]

As shown in Figure 4E, the most common treatment for patients in the included studies was conventional surgery (CS; n = 16; 66.67%).[171, 235-245] The remaining treatments were minimally invasive surgeries, including laparoscopic (n = 5; 20.83%),[244, 245, 248-250] robot-assisted surgery (RAS; n = 2; 8.33%),[238, 248] and video-assisted thoracoscopic surgery (VATS; n = 1; 4.17%).[237] Postoperative follow-up times varied across publications. In all the included publications, the farthest follow-up time-point was one year after surgery. To unify the results postoperative time-points were divided into the following three time-frames: within 7 days after surgery (n = 10; 58.82%),[236-238, 240, 241, 244, 247-250] between 7 days and 3 months after surgery (n = 6; 35.30%),[171, 235, 239, 242, 245, 246] or between 3 months to 1 year after surgery (n = 1; 5.88%).[243] This data is depicted in Figure 4F.

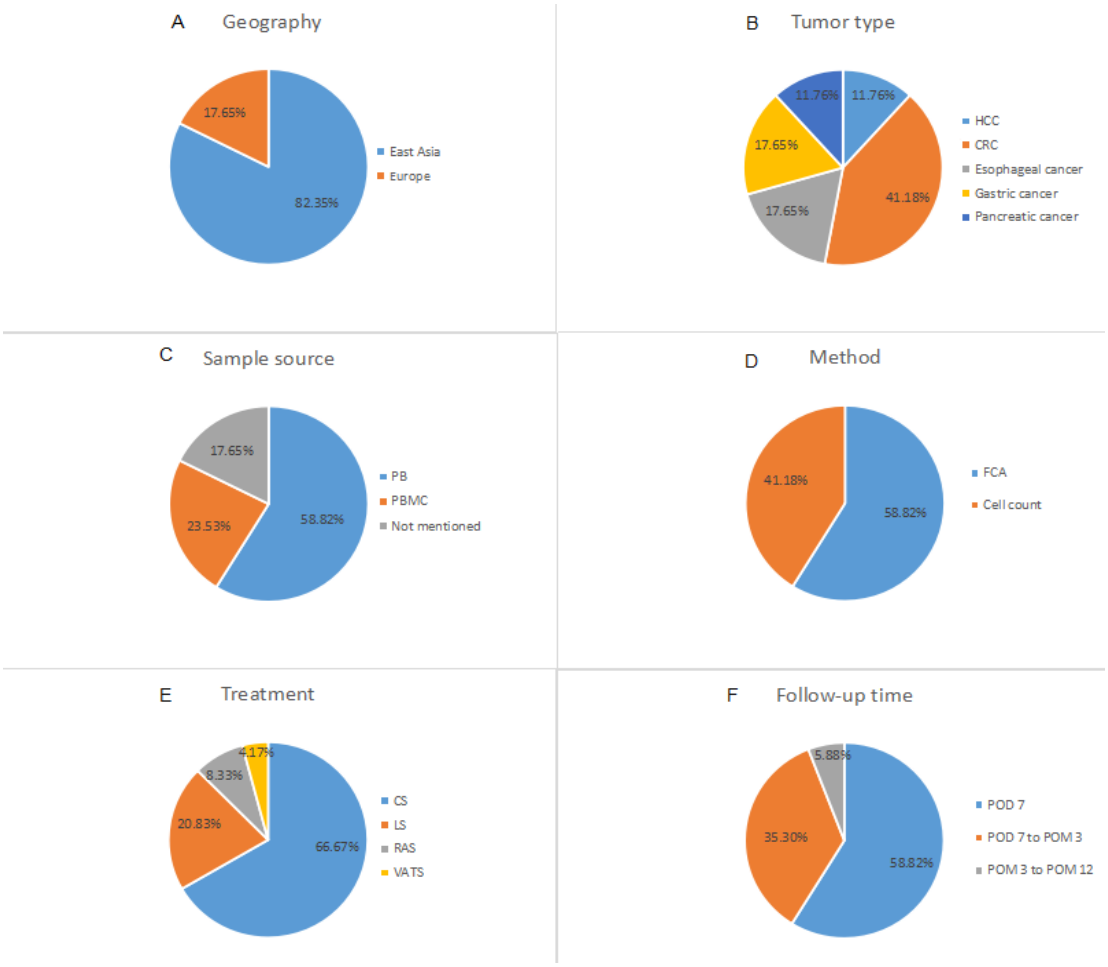

Figure 4. Characteristics of studies included in the review. (A): Geographic distribution of publications; (B): Classification of digestive system tumors; (C): Source of the specimen; (D): Detection methods; (E): Surgical methods; (F): Postoperative follow-up time. (Abbreviations: CRC: Colorectal cancer; HCC: Hepatocellular carcinoma; PB: Peripheral blood; PBMC: Peripheral blood mononuclear cells; FCA: Flow cytometry analysis; CS: Conventional surgery; LS: Laparoscopic; RAS: Robot-assisted surgery; VATS: Video-assisted thoracoscopic surgery; POD: Postoperative day; POM: Postoperative month).

The following paragraphs will systematically describe the postoperative progression of subsets of circulating immune cells in patients with digestive system tumors and their correlation with clinical characteristics and outcome data.

Seven publications reported changes in perioperative peripheral WBC (also called leukocytes) counts. The overall WBC counts were increased on postoperative day

(POD) 1, then gradually decreased. In most of the included studies, WBC counts returned to the preoperative level by POD 7.[242, 244-246, 248-250] However, Maas et al. reported that in esophageal cancer patients, WBC counts remained elevated in the CS group compared to those in the minimally invasive surgery group at POD 7. This may be attributed to the fewer postoperative respiratory infections observed in the minimally invasive surgery group.

Four studies provided information regarding overall lymphocyte counts. Compared with preoperatively, lymphocyte counts were significantly reduced on POD 1, gradually returning to the preoperative level within 30 days after the operation.[245-247] Fujii et al. demonstrated that HLA-DR$^+$ lymphocyte counts (defined in this publication as activated lymphocytes) were nearly restored to preoperative levels on POD 7; however, the overall lymphocyte count remained low in 20 gastric cancer patients.[244]

As a systemic inflammatory response marker, the NLR has the advantage of being easily obtained from routine laboratory tests of PB cell counts. Changes in the perioperative NLR may reflect changes in the systemic inflammatory response and immune response balance post-surgery.[171] As shown in Supplementary Table 1, three publications calculated the NLR perioperatively. All three used different methods for triangulating the perioperative inflammatory response measured by the NLR. One study by Peng et al. calculated the ΔNLR (postoperative NLR − preoperative NLR = ΔNLR). The groups were divided into decreasing and increasing NLR. Patients with a decreasing NLR post-surgery showed significantly better OS and RFS (date of resection to recurrence or death from any cause).[171] Miyatani et al. reported 280 gastric cancer patients who underwent CS and calculated their NLR both preoperatively and within 3 months after the operation. Their results showed that neither preoperative nor postoperative NLR alone was able to predict patient survival following a gastrectomy. However, the five-year OS rates of patients with both low

preoperative and postoperative NLR was significantly improved than those with high preoperative and/or postoperative NLR. After analysis of the area under the curve, poor yet significant predictability was shown.[235] Kubo et al. measured the NLR at 3 time-points within 7 days perioperatively (preoperatively, POD 1, and POD 3) in 524 CRC patients. Based on their NLR, patients were divided into a high NLR group (high NLR at > 1 time point) and a low NLR group (high NLR at ≤ 1 time point). The results revealed that increased perioperative NLR was a risk factor for survival rate following curative resection.[241]

As seen in Supplementary Table 1, a total of nine studies investigated T cells (which have the function of assisting other lymphocytes to exert immunological activity, killing function, and immunosuppressive function). Three of them observed a significant reduction in T cell (defined as $CD3^+$)[237, 244, 245] counts and activated T cell (defined in one publication as $CD3^+HLA\text{-}Dr^+$)[245] counts following surgery. All the above-mentioned three reports that counts returned to preoperative level about one week after surgery (POD 7). Wang et al. showed that in contrast with pre-operation (PreOP), there was no statistical difference in the number of T lymphocytes in 7 CRC patients on POD 7.[236]

Six reports described $CD4^+$ (defined as Th, not only can help macrophages eliminate pathogenic microorganisms, activate B lymphocytes to secrete antibodies, and can kill infected target cells with the help of aCTL) cells and $CD8^+$ (defined as CTL, which are crucial immune cells for tumor surveillance and immune defense against intracellular pathogenic microorganism, to kill malignant or infected cells) cells. Most reports (66.67%) showed that these two kinds of cells decreased on the first POC compared with preoperative levels, and gradually recovered to preoperative level on postoperative one week[237, 238, 244, 245, 249] or 12 months,[243] and the ratio of $CD4^+/CD8^+$ cell was similar to the trend described above.[237] However, Ordemann et al. reported

no significant change on POD 7 compared with preoperative levels.[249]

As shown in Supplementary Table 1, only one study mentioned Tregs. Chen et al. found that the counts of a group of Tregs (defined as $CD4^+CD25^+CD127^-$) dramatically increased after resection in 36 HCC patients. This effect was highest on POD 7, but they did not report when it recovered to the preoperative level.[247]

Besides, Ling et al. reported that the levels of Th17 (defined as $IL\text{-}17^+IL\text{-}22^-IFN\text{-}\gamma^-CD4^+$ in this publication, which is considered pro-inflammatory because they can produce IL-17, also can against host defense infection by recruiting Inflammatory cells to infected tissues), Th22 (defined as $IL\text{-}17^-IL\text{-}22^+IFN\text{-}\gamma^-CD4^+$, which are closely related to many kinds of diseases, including tumor, autoimmune diseases, and inflammations) and $IL\text{-}17^+IL\text{-}22^+IFN\text{-}\gamma^-CD4^+$ T cells were higher than preoperatively in 31 CRC on POD 14. However, the percentage of Th1 cells (which not only can produce TNF-β, IL-2, and IFN-γ but also activate macrophages and are in charge of cell-mediated immunity) in CRC patients before and after surgery was similar, with no significant difference found.[239]

Four studies reported on B cells, whose primary function is to secrete antibodies that mediate fluid immune responses. Shibata et al. perioperatively measured B cell levels (defined as $CD3^-/CD19^+$) in 46 CRC patients at four time-points over 7 days (preoperatively, POD 1, POD 3, and POD 6). They found there was no significant change in B cell levels after resection compared to preoperative levels.[238] Similarly, Leung et al. measured B cell levels in 40 rectosigmoid carcinoma patients at four time-points (preoperatively, POD 1, POD 3, and POD 8) and found no significant difference between preoperative and postoperative levels.[245]

Two studies reported on Bregs. They can promote the development of Tregs and inhibit eTh and CTL by secreting IL-10. One study by Shi et al. demonstrated no significant

difference in the percentage of Bregs (defined as $CD5^+CD19^+$ cells) between 60 patients with esophageal cancer before CS and POD 1. However, a significant reduction of Bregs was observed in esophageal cancer patients seven days after tumor resection compared with the counts before the surgery and POD 1.[240] Chen et al. also examined Bregs (defined as $CD19^+IL\text{-}10^+$ cells) pre and postoperatively in 36 HCC patients. However, unlike Shi et al.'s study, the frequency of peripheral Bregs was found to have significantly increased following tumor resection in all patients, particularly on POD 7.[247] The authors of both studies, at first glance show opposing results. From Chen et al. described in the discussion, the increase in postoperative Bregs can promote tumor metastasis and recurrence in HCC patients, due to inhibition of immunity after resection.[247]

Three studies showed that POD 1 levels of NK cells,[237, 238] which can directly kill tumor cells or virus-infected lymphocytes and possess both cytotoxic and immunoregulatory functions, were lower in postoperative PB measurements compared to preoperative samples. Additionally, NK cell subsets, including non-major histocompatibility complex (MHC)-restricted NK cells (defined as $CD3^-CD16^+CD56^+$) and MHC-restricted NK-like cells (defined as $CD3^+CD16^+CD56^+$),[245] also exhibited lower postoperative levels following PB measurement. However, all cell counts returned to near preoperative level on approximately POD 7. Fujii et al. reported similar changes of $CD57^+$ cell counts (defined as activated NK cells) among 20 gastric cancer patients.[244] Takahashi et al. demonstrated no significant differences in NK cell counts (defined as $CD14^-/CD56^+$) on postoperative month (POM) 12 compared to preoperative levels in 20 pancreatic cancer patients.[243] Besides this, Wang et al. reported the percentages of NK lymphocytes and the percentages of NKT at 2 time-points (preoperatively and POD 7) in 7 CRC patients. Results displayed that there was no different significance on POD 7 compared with preoperatively measured levels.[236]

DC is the most potent APC which can express co-stimulatory molecules and higher MHC. Takahashi et al. divided DC into two functionally heterogeneous subgroups: DC1 ($CD11c^+$ DCs, known as myeloid DC population, which a major part through intense stimulation of naive T cells to protect against cancer) and DC2 ($CD11c^-$ DCs, known as lymphoid DC population, which stimulate allogeneic naive T cells with CD2 can make Th2 cells generated IL-4, some report mentioned Th1 responses can be stimulated by DC2). As depicted in Supplementary Table 1, the cDC1 cell (circulating myeloid DC1s) count and the cDC1:cDC2 (circulating myeloid DC2s) ratios of 20 resected pancreatic cancer patients increased 12 months postoperatively. However, no significant change in cDC2 cell counts was observed between pre- and postoperative levels. This article concluded when pancreatic cancer patients did not have recurrence and metastasis, the cDC1 count, and the ratio of cDC1/cDC2 back to normal 1 year after surgery.[243]

1.10. Aim of this Study

This study is an exploration of the changes in immune patterns before and after surgery of non-HBV/HCV HCC patients. We aimed at correlating these results with clinicopathological variables and compared the longitudinal patterns of recurrence and non-recurrence patients.

2. Material and Methods

2.1. Materials

2.1.1. Laboratory Equipment

Centrifuge	Rotina 380R, Hettich, Germany
Combitips Plus	PD-Tips, Brand, Germany
Electronic balance	MP-3000, Waagen dienst, Germany
Flow Cytometer	LSRFortessa™, BD Biosciences, USA
Multipette Plus	HandyStep® S, Brand, Germany
Pipettes	Transferpette® S, Brand, Germany
Vortex	G560E, Scientific Industries, USA
4℃ fridge	FKS 5000, Liebherr, Germany

2.1.2. Computer and Software

Computer hardware	Z230 SFF Workstation, HP, USA
FACSDIVA™ SOFTWARE	BD, USA
Prism	Version 8.0.2, GraphPad Software, USA
SPSS	Version 21.0, USA

2.1.3. Consumables

0.5-20µL, Ep T.I.P.S.® Reloads	Eppendorf, Germany
2-200µL, Ep T.I.P.S.® Reloads	Eppendorf, Germany
Electronic pipette	Accu-iet® pro, Brand, Germany
Gloves	Eco Nitrile PF 250, EvoShield, USA
7.5ml Lithium Heparin blood collection tube	S-Monovette®, Sarstedt, USA
5ml Polystyrene Round-Bottom Tube	12×75mm style, Falcon, USA
10ml Stripette	Corning, USA

2.1.4. Chemical

Bovine Serum Albumin (BSA), Fraction V	Biomol, Germany
Natriumazid 10%	Morphisto, Germany
Millipore H_2O	Advantage A10, Merck, Germany
FACS™ Lysing Solution	BD, USA
Golgi Stop	BD, USA
IC Fixation buffer(10x)	Invitrogen, USA
Permeabilization buffer(10x)	Invitrogen, USA

2.1.5. Buffers and Solutions

1x Lysing Solution	pH	7.1
	50ml	10x Lysing Solution

	450ml	Millipore H_2O
1x Permeabilization buffer	pH	7.3
	8ml	10x Permeabilization buffer
	72ml	Millipore H_2O
FACS buffer	pH	7.4
	1 L	1x DPBS
	2ml	Natriumacid
	5g	BSA

2.1.6. Antibodies

Antibody	**Isotype**	**Fluorochrome**	**Reactivity**	**Clone**
Anti-CD3	Mouse (BALB/c) IgG1, κ	PerCP Cy5.5	Human	
Anti-CD4	Mouse (BALB/c) IgG1, κ	BUV395	Human	SK3
Anti-CD5	Mouse (BALB/c) IgG1, κ	BV421	Human	UCHT2
Anti-CD8	Mouse (BALB/c) IgG1, κ	APC-H7	Human	SK1
Anti-CD10	Mouse (BALB/c) IgG1, κ	PE	Human	HI10a
Anti-CD14	Mouse BALB/c IgG_{2b}, κ	BV510	Human	MyP9
Anti-CD15	Mouse IgG1, κ	PE-CF594	Human	W6D3
Anti-CD16	Mouse BALB/c IgG1, κ	FITC	Human	B73.1
Anti-CD19	Mouse (BALB/c) IgG1, κ	FITC	Human	HIB19
Anti-CD20	Mouse BALB/c IgG_{2a}, κ	APC-H7	Human	H1
Anti-CD24	Mouse BALB/c IgG_{2a}, κ	PE-CF594	Human	MLS

Anti-CD25	Mouse (BALB/c) IgG1, κ	BB515	Human	2A3
Anti-CD27	Mouse (BALB/c) IgG1, κ	BV786	Human	L128
Anti-CD33	Mouse BALB/c IgG1, κ	BV786	Human	WM53
Anti-CD38	Mouse (BALB/c) IgG1, κ	BV605	Human	HB7
Anti-CD45	Mouse (BALB/c) IgG1, κ	BV650	Human	HI30
Anti-CD56	Mouse BALB/c IgG2b, κ	APC R700	Human	NACM1 6.2
Anti-CD68	Mouse BALB/c IgG2b, κ	BV711	Human	n.a
Anti-CD69	Mouse IgG1, κ	BUV395	Human	FN50
Anti-CD127	Mouse IgG1, κ	PE-CF594	Human	HIL-7R-M21
Anti-CD194	Mouse C57BL/6 IgG1, κ	BV510	Human	SK3
Anti-CD196	Mouse IgG1, κ	PE	Human	11A9
Anti-CD197	Mouse IgG2a	BV421	Human	150503
Anti-CD1d	Mouse (BALB/c) IgG1, κ	APC	Human	CD1d42
Anti-CD11b	Mouse IgG1, κ	PECy7	Human	ICRF44
Anti-CD11c	Mouse (BALB/c) IgG1, κ	PE	Human	B-ly6
Anti-CD45RO	Mouse (BALB/c) IgG2a, κ	PE-Cy7	Human	UCHL1
Anti-CD66b	Mouse BALB/c IgM, κ	Alexa 647	Human	G10F5
Anti-IgD	Mouse BALB/c IgG2a, κ	PE-Cy7	Human	IA6-2
Anti-IgM	Mouse (BALB/c) IgG1, κ	BV510	Human	G20-127
Anti-HLA-DR	Mouse IgG2a, κ	APC	Human	G46-6
Anti-HLA-DR	Mouse IgG2a, κ	BV421	Human	G46-6

2.2. Methods

2.2.1. Patients and Samples

In our study, HCC patients who are meeting the following criteria were included: 1) non-HBV/HCV patients; 2) older than 18 years and able to consent to participate in this study; 3) who are listed for LT or are due for resection. Exclude criteria were as follows: 1) refuse to participate in our study; 2) younger than 18 years or unable to consent; 3) patients were pregnant; 4) suffering from hepatitis or another infectious disease; 5) has a synchronous second malignancy; 6) had undergone invasive liver surgery (biopsy, operative sampling, TACE, RFA, etc) 6 weeks before initial blood sampling. All patients received curative LR between 2016 and 2019 at the Department of General, Visceral, Transplantation Surgery of Ludwig-Maximilians-University Munich hospital. PB specimens were gathered at preoperative one day, post-operative three months, and one year after surgery, respectively, and samples were processed within 24 hours. No patients received any anti-cancer therapy before sampling. The study was approved by the Institutional Review Board (EK54-16) of the Ludwig-Maximilians-University Hospital and informed consent was acquired from all patients for the blood specimen collection.

2.2.2. Immune Cells and Cluster of Differentiation Marker

Different immune cells have specific immunophenotyping. Based on cell lineage and developmental stage, cell subsets can be defined by cell surface markers, labeled with antibodies, and use flow cytometry (FCM) for analysis. The study included the following immune cells, as shown in Table 2.

Table 2. Definition of cell subtypes. Definition of cell subtypes. Abbreviations: aCTL: Activited cytotoxic T lymphocytes; aTh: Activited helper T cells; aTregs: Activited

regulatory T cells; Bregs: Regulatory B cells; CD: Cluster of differentiation; cmCTL: Central memory cytotoxic T lymphocytes; cmTh: Central memory helper T cells; cs-memory B cells: Class-switched memory B cells; ns-memory B cells: Non-class-switched memory B cells; CTL: Cytotoxic T lymphocytes; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; eTh: Effector helper T cells; mTregs: Memory regulatory T cells; maTregs: Memory activated regulatory T cells; MDSC: Myeloid-derived suppressor cells; G-MDSC: Granulocyte-like MDSC; M-MDSC: Monocyte-like MDSC; nCTL: Naïve cytotoxic T lymphocytes; NK cells: Natural killer cells; NKT cells: Natural killer T cells; nTh: Naïve helper T cells; nTregs: Naïve regulatory T cells; Pre B cells: Precursor B cells; Pro B cells: Progenitor B cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells; Tregs: Regulatory T cells.

Cell type	**Marker**
T cells, % of Leukocytes[251]	$CD45^+/CD3^+$, % of $CD45^+$
Th, % of T cells[252]	$CD45^+/CD3^+/CD4^+/CD8^-$, % of $CD45^+/CD3^+$
Th1, % of Th[253]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR4^-/CCR6^-$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
Th2, % of Th[253]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR4^+/CCR6^-$, % of $CD45^+/CD3^+/CD4^+/CD8^-$

Th17, % of Th[253]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR4^+/CCR6^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
emTh, % of Th[254]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR7^-/CD45RO^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
cmTh, % of Th[254]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR7^+/CD45RO^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
eTh, % of Th[255]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR7^-/CD45RO^-$, % of $CD45^+/CD3^+/CD4^+/CD8$
nTh, % of Th[256, 257]	$CD45^+/CD3^+/CD4^+/CD8^-/CCR7^+/CD45RO^-$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
aTh, % of Th[258]	$CD45^+/CD3^+/CD4^+/CD8^-/HLA\text{-}DR^+/CD38^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-$
CTL, % of T cells[259]	$CD45^+/CD3^+/CD8^+/CD4^-$, % of $CD45^+/CD3^+$
emCTL, % of CTL[260]	$CD45^+/CD3^+/CD8^+/CD4^-/CCR7^-/CD45RO^+$, % of $CD45^+/CD3^+/CD8^+/CD4^-$
cmCTL, % of CTL[261]	$CD45^+/CD3^+/CD8^+/CD4^-/CCR7^+/CD45RO^+$, % of $CD45^+/CD3^+/CD8^+/CD4^-$
eCTL, % of CTL[262]	$CD45^+/CD3^+/CD8^+/CD4^-/CCR7^-/CD45RO^-$, % of $CD45^+/CD3^+/CD8^+/CD4^-$

nCTL, % of CTL[263, 264]	$CD45^+/CD3^+/CD8^+/CD4^-/CCR7^+/CD45RO^-$, % of $CD45^+/CD3^+/CD8^+/CD4^-$
aCTL, % of CTL[265]	$CD45^+/CD3^+/CD8^+/CD4^-/HLA\text{-}DR^+/CD38^+$, % of $CD45^+/CD3^+/CD8^+/CD4^-$
Tregs, % of Th[266]	$CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-$, % of $CD45^+/CD3^+CD4^+/CD8^-$
mTregs, % of Tregs[267, 268]	$CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-/HLA\text{-}DR^-/CD45RO^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-$
nTregs, % of Tregs[267]	$CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-/HLA\text{-}DR^-/CD45RO^-$, % of $CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-$
aTregs, % of Tregs[269, 270]	$CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-/HLA\text{-}DR^+/CD45RO^-$, % of $CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-$
maTregs, % of Tregs[271]	$CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-/HLA\text{-}DR^+/CD45RO^+$, % of $CD45^+/CD3^+/CD4^+/CD8^-/CD25^+/CD127^-$
B cells, % of Leukocytes[272]	$CD45^+/CD19^+/CD3^-$, % of $CD45^+$
Memory B cells, % of B cells[228, 273]	$CD45^+/CD19^+/CD3^-/CD27^+$, % of $CD45^+/CD19^+/CD3^-$
cs-memory B cells, % of B	$CD45^+/CD19^+/CD3^-/CD27^+/IgD^-/IgM^-$

cells[274, 275]	/CD20^{+}/CD38^{+-}, % of CD45^{+}/CD19^{+}/CD3^{-}
Plasmablasts, % of B cells[276]	CD45^{+}/CD19^{+}/CD3^{-}/CD27^{+}/IgD^{-}/IgM^{-}/CD20^{-} /CD38^{hi}, % of CD45^{+}/CD19^{+}/CD3^{-}
ns-memory B cells, % of B cells[228, 274]	CD45^{+}/CD19^{+}/CD3^{-}/CD27^{+}/ IgD^{+}, % of CD45^{+} /CD19^{+}/CD3^{-}
Naive B cells, % of B cells	CD45^{+}/CD19^{+}/CD3^{-}/CD27^{-}/IgD^{+}, % of CD45^{+}/CD19^{+}/CD3^{-}
Transitional B cells, % of B cells[277, 278]	CD45^{+}/CD19^{+}/CD3^{-}/CD24^{hi}/CD38^{hi}, % of CD45^{+}/CD19^{+}/CD3^{-}
Bregs-1, % of Transitional B cells[279, 280]	CD45^{+}/CD19^{+}/CD3^{-} /CD24^{hi}/CD38^{hi}/CD1d^{+}/CD5^{+}, % of CD45^{+}/CD19^{+}/CD3^{-}/CD24^{hi}/CD38^{hi}
Pro B cells, % of Transitional B cells[281, 282]	CD45^{+}/CD19^{+}/CD3^{-} /CD24^{hi}/CD38^{hi}/CD10^{+}/IgM^{-}, % of CD45^{+}/CD19^{+}/CD3^{-}/CD24^{hi}/CD38^{hi}
Pre B cells, % of Pro B cells[282, 283]	CD45^{+}/CD19^{+}/CD3/CD24^{hi}/CD38^{hi}/CD10^{+}/IgM^{-} /CD20^{+}, % of CD45^{+}/CD19^{+}/CD3^{-} /CD24^{hi}/CD38^{hi}/CD10^{+}/IgM^{-}
Plasma cells, % of B cells[284]	CD45^{+}/CD19^{+}/CD3^{-}/CD10^{-}/IgD^{-}/IgM^{-} /CD27^{hi}/CD38^{hi}, % of CD45^{+}/CD19^{+}/CD3^{-}
Plasma cells 1, % of B cells[285]	CD45^{+}/CD19^{+}/CD3^{-}/CD10^{-}/CD27^{hi}/CD38^{hi}, % of

	$CD45^+/CD19^+/CD3^-$
Neutrophils, % of Leukocytes[286, 287]	$CD45^+/CD66b^+/CD15^+$, % of $CD45^+$
Monocytes, % of Leukocytes[288, 289]	$CD45^+/CD14^+/CD33^+$, % of $CD45^+$
Macrophages, % of Leukocytes[290, 291]	$CD45^+/CD33^+/CD11b^+/CD11c^+/CD8^+$, % of $CD45^+$
DC, % of Leukocytes[292, 293]	$CD45^+/CD33^+/HLA\text{-}DR^+/CD11c^+/CD11b^-$, % of $CD45^+$
MDSC, % of Leukocytes[294, 295]	$CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+$, % of $CD45^+$
G-MDSC, % of Leukocytes[194]	$CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+/CD15^+/CD14^-$, % of $CD45^+$
G-MDSC, % of MDSC[296]	$CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+/CD15^+/CD14^-$, % of $CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+$
M-MDSC, % of Leukocytes[295, 297]	$CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+/CD14^+/CD15^-$, % of $CD45^+$
M-MDSC, % of MDSC[297]	$CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+/CD14^+/CD15^-$, % of $CD45^+/HLA\text{-}DR^-/CD11b^+/CD33^+$

NK cells, % of Leukocytes[298]	$CD45^+/CD3^-/CD16^+/CD56^+/CD8^{+-}$, % of $CD45^+$
$CD69^+$ NK cells, % of NK cells[299]	$CD45^+/CD3^-/CD16^+/CD56^+/CD8^{+-}/CD69^+$, % of $CD45^+/CD3^-/CD16^+/CD56^+/CD8^{+-}$
NKT cells, % of Leukocytes[300]	$CD45^+/CD3^+/CD16^+/CD56^+/CD8^{+-}$, % of $CD45^+$
$CD69^+$ NKT cells, % of NKT cells[301]	$CD45^+/CD3^+/CD16^+/CD56^+/CD8^{+-}/CD69^+$, % of $CD45^+/CD3^+/CD16^+/CD56^+/CD8^{+-}$

2.2.3. Staining Panels

The immune cell is labeled according to the CD expressed by the cell to select the corresponding antibody. We divided the staining panels into T cells and their subtypes (Supplementary Table 2), B cells and their subtypes (Supplementary Table 3), neutrophils, monocytes, macrophages, DC, as well as NK, NKT, MDSC, and their respective subtypes (Supplementary Table 4). Each panel contained an unstained tube for blank control, fluorescence minus one (FMO) for gating, and a corresponding sample tube. The antibodies were sequentially added to the corresponding tubes according to the staining panel, and the amounts of each antibody are shown in Supplementary Tables 2 to 4.

2.2.4. Immunophenotyping Staining Protocol of Peripheral Whole Blood for HCC Patients

On the day of LR of patients with primary HCC, 3 months after the operation and 1 year after surgery, Lithium Heparin blood collection tubes were used to collect a maximum of 15ml whole blood samples for measurement, respectively. Samples were measured immediately or at least 24h after collection. The detailed steps of cell

staining were as follows：

T cell staining protocol in whole blood (extracellular staining): All steps were performed at room temperature. We added 200ul whole blood to every florescence activating cell sorting (FACS) tube, then the corresponding amount of antibody was added according to the T cell staining panel (Supplementary Table 2). After the vortex, all tubes were incubated in the darkroom for 15 minutes, then added 2ml of 1x FACS lysing solution to all tubes, and incubate for 10 minutes in the darkroom after a gentle vortex. The supernatant was discarded after centrifugation at 500xg for 5 minutes. Vortex after adding 2ml of FACS buffer for every tube and the supernatant was discarded after centrifugation at 500xg for 5 minutes. Finally, we added 200ul of FACS buffer to every tube and then measured it.

B cells, neutrophils, monocytes, macrophages, DC, NK cells, NKT cells, and MDSC staining protocols in whole blood (intracellular staining): All steps were performed at room temperature. We added 200μl of whole blood to every FACS tube, then according to the staining panels (Supplementary Table 3 and 4), the corresponding amount of antibody was added to each tube (except CD20 and CD68). Incubate all tubes in the darkroom for 15 minutes, then added 2ml of 1x FACS lysing solution to each tube and incubated for 10 minutes in the darkroom after vortex. Discarded the supernatant after centrifugation at 500xg for 5 minutes. Added 100ul of IC fixation buffer to each tube, and vortexed, incubate in the darkroom for 20 minutes. Then added 2ml of 1x perm buffer to all tubes, and centrifuged 500xg for 5 minutes after the vortex, then discarded the supernatant (repeat this step once). Add intracellular antibodies CD20 and CD68 to tubes according to the respective panels, and incubate all tubes for 30 minutes in the darkroom after shaking. Added 2ml of 1x perm buffer to each tube, then vortex and centrifuge 500xg for 5 minutes, discarded the supernatant, and added 2ml FACS buffer. After vortex and centrifuge 500xg for 5 minutes, all tubes discarded the supernatant

and finally added 200ul FACS buffer for measurement.

2.2.5. Gating Strategy

The basis for setting the gate is depending on the unstained cells and FMOs. The detailed gating strategy flow chart is as follows:

T cell panel

Figures 5A and 5B show dot plots of all immune cells and leukocytes (defined as $CD45^+$), and further divided into $CD45^+/CD3^+$ T lymphocytes (Figure 5C). Based on $CD3^+$ T lymphocytes, it is divided into CTL (defined as $CD3^+/CD4^-/CD8^+$) and Th (defined as $CD3^+/CD4^+/CD8^-$) (Figure 5D). CTL can be further differentiated into activated CTL (aCTL, defined as HLA-$DR^+/CD38^+$) (Figure 5E). As shown in Figure 5F, according to the expression of CD45RO and CD197, CTL also can be divided into again the following four subsets: naive CTL (nCTL, defined as $CD197^+/CD45RO^-$), effector CTL (eCTL, defined as $CD197^-/CD45RO^-$), effector memory CTL (emCTL, defined as $CD197^-$/ $CD45RO^+$) and central memory CTL (cmCTL, defined as $CD197^+/CD45RO^+$). Th can be further partitioned into Th1 ($CD194^-/CD196^-$), Th2 ($CD194^+/CD196^-$), Th17 ($CD194^+/CD196^+$) (Figure 5G), and activated Th (aTh, defined as HLA-$DR^+/CD38^+$) (Figure 5H). According to the expression of CD197 and CD45RO, Th can be divided into four different developmental stages: naive Th (nTh, defined as $CD197^+/CD45RO^-$), effector Th (eTh, defined as $CD197^-/CD45RO^-$), effector memory Th (emTh, defined as $CD197^-/CD45RO^+$), central memory Th (cmTh, defined as $CD197^+/CD45RO^+$) (Figure 5I). From Figure 5J, Th can be divided into Tregs ($CD25^+/CD127^-$), and four subtypes: naive Tregs (nTregs, defined as HLA-DR^- /$CD45RO^-$), activated Tregs (aTregs, defined as HLA-$DR^+/CD45RO^-$), memory Tregs (mTregs, defined as HLA-$DR^-/CD45RO^+$), memory activated Tregs (maTregs, defined as HLA-$DR^+/CD45RO^+$) (Figure 5K).

B cell panel

$CD45^+$ leukocytes (Figure 6B) were determined based on clustering (Figure 6A). $CD3^-$/$CD19^+$ B cells cluster were gated based on the markers of B cells (Figure 6C). B cells are divided into $CD24^{hi}$/$CD38^{hi}$ transitional B cells (Figure 6D), and a subset thereof: $CD1d^+$/$CD5^+$ Bregs-1 (Figure 6E), $CD10^+$/IgM^- pro B cells and $CD10^+$/IgM^-/$CD20^+$ pre B cells (Figure 6F and G). Based on whether CD27 is expressed, B cells can be partitioned into $CD27^+$ and $CD27^-$ B cells (Figure 6H), which can be further divided into IgD^+ n-s memory B cells (Figure 6I). IgD^-/IgM^- can be divided into again $CD20^-$/$CD38^{hi}$ plasmablasts and $CD20^+$/$CD38^{+-}$ c-s memory B cells (Figure 6J). $CD27^-$ can be further partitioned into IgD^+ naive B cells (Figure 6K). $CD10^-$ B cells (Figure 6L) can be divided into IgD^-/IgM^- (Figure 6M) and its subset $CD27^{hi}$/$CD38^{hi}$ plasma cells (Figure 6N). Separate $CD27^{hi}$/$CD38^{hi}$ plasma cells 1 from $CD10^-$ (Figure 6O).

Neutrophils, monocytes, macrophages, DC, NK, NKT, and MDSC panel

$CD45^+$ leukocytes (Figure 7B) were gated based on clustering (Figure 7A). $HLA\text{-}DR^-$ (Figure 7C) was gated form leukocytes and further divided into $CD11b^+$/$CD33^+$ MDSC (Figure 7D). As shown in Figure 7E, MDSC can be further divided into again $CD15^+$/$CD14^-$ G-MDSC and $CD15^-$/$CD14^+$ M-MDSC. As shown in Figure 7F and G, $CD15^+$/$CD66b^+$ neutrophils and $DC14^+$/$CD33^+$ monocytes, both of them were divided from $CD45^+$ leukocytes. Also, $CD33^+$ (Figure 7H) can be divided into two branches: one is $CD11b^+$/$CD11c^+$ and its subsets: $CD68^+$ macrophages (Figure 7I); the other one is $HLA\text{-}DR^+$ (Figure 7J) and its subtypes: $CD11b^-$/$CD11c^+$ DC (Figure 7K). From Figure 7L, we can see that $CD45^+$ leukocytes can be partitioned into the following parts: $CD16^+$/$CD3^-$ and $CD16^+$/$CD3^+$, $CD16^+$/$CD3^-$ can be further divided into $CD56^+$/$CD8^{+-}$ NK cells and its activated subtype: $CD69^+$ NK cells (Figure 7M and N); also, $CD16^+$/$CD3^+$ can be further divided into $CD56^+$/$CD8^{+-}$ NKT cells and its activated

subtype: $CD69^+$ NKT cells (Figure 7O and P).

2.2.6. Statistical Analysis

Measurement data were expressed as means ± standard deviations (SD). The SPSS 21.0 software package was used for statistical analysis. The residual plot method was used to test whether the measurement variables were subject to a normal distribution. Statistical significance was analyzed by one-way repeated-measures ANOVA. Bonferroni correction was used to account for multiple testing. This was performed by dividing αBC (before correction = 0.05) by the number of comparisons (K). A newly calculated threshold of αAC (after correction) was required to achieve significance. Forty-nine variables were measured and compared. Therefore, the corrected αAC threshold was calculated as 0.05/49, giving 0.00102. A p-value of < 0.001 was considered statistically significant. For comparison between multiple time points, Bonferroni's multiple comparison test was used to compare multiple independent groups. The correlation between immune cells and pre-surgery clinical parameters was described in two parts. A Mann-Whitney U test and one-way ANOVA were used to examine the relationship between contingency and continuous data and presented as p values. Pearson's correlation coefficient has examined the correlations between two continuous data and showed as r and p values. $P < 0.05$ were deemed statistically significant differences.

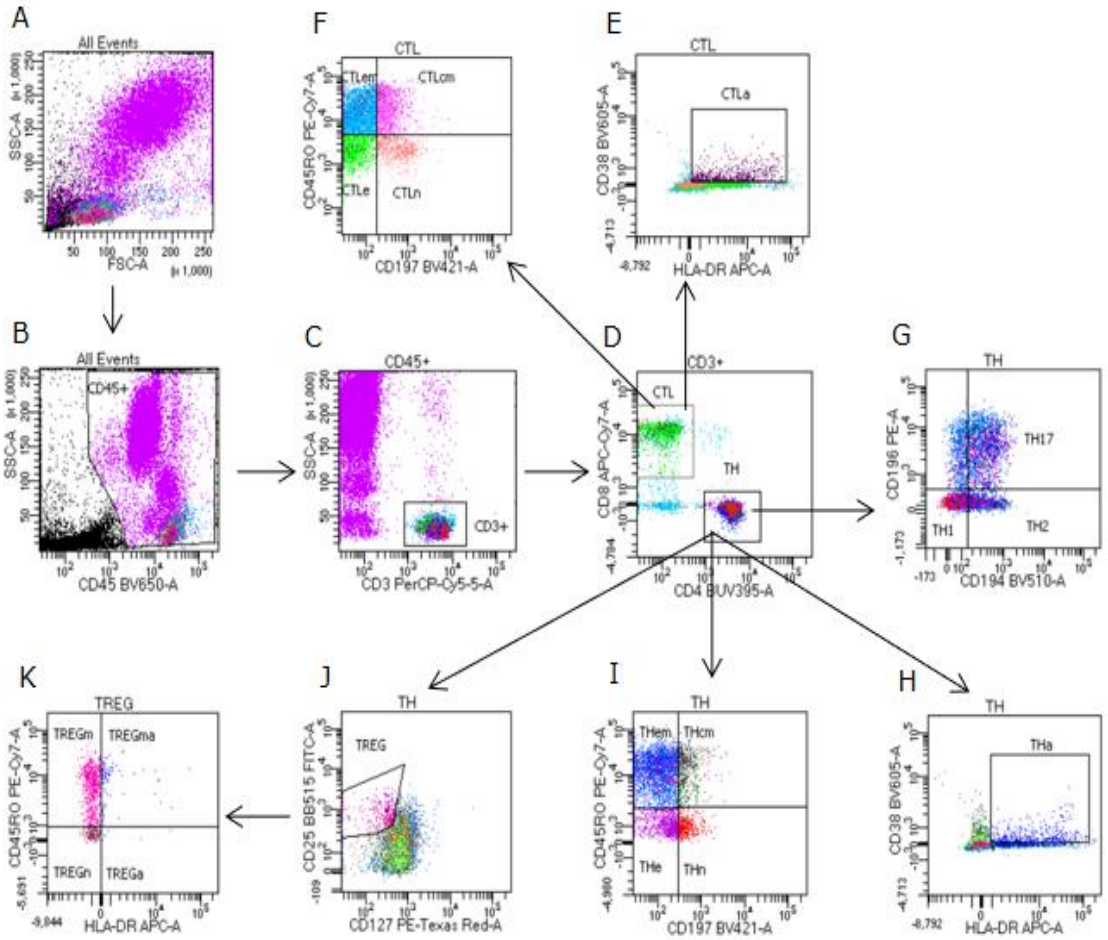

Figure 5. Gating strategies for T cells and their subtypes. (A): All events display; (B): CD45+ leukocytes; (C): CD45+/CD3+ T lymphocytes; (D): CD3+ T lymphocytes were divided into Th (CD4+/CD8-) and CTL (CD4-/CD8$^+$); (E): HLA-DR$^+$/CD38$^+$ aCTL; (F): According to the expression of CD45RO and CD197, CTL was divided into: CD197$^+$/CD45RO$^-$ nCTL, CD197$^-$/CD45RO$^-$ eCTL, CD197$^-$/CD45RO$^+$ emCTL and CD197$^+$/CD45RO$^+$ cmCTL; (G-J): Th were divided into the following subsets: CD194$^-$/CD196$^-$ Th1, CD194$^+$/CD196$^-$ Th2, CD194$^+$/CD196$^+$ Th17, HLA-DR$^+$/CD38$^+$ aTh, CD197$^+$/CD45RO$^-$ nTh, CD197$^-$/CD45RO$^-$ eTh, CD197$^-$/CD45RO$^+$ emTh, CD197$^+$/CD45RO$^+$ cmTh and CD25$^+$/CD127$^-$ Tregs; (K): Four subsets of Tregs: HLA-DR$^-$/CD45RO$^-$ nTregs, HLA-DR$^+$/CD45RO$^-$ aTregs, HLA-DR$^-$/CD45RO$^+$ mTregs and HLA-DR$^+$/CD45RO$^+$ maTregs.

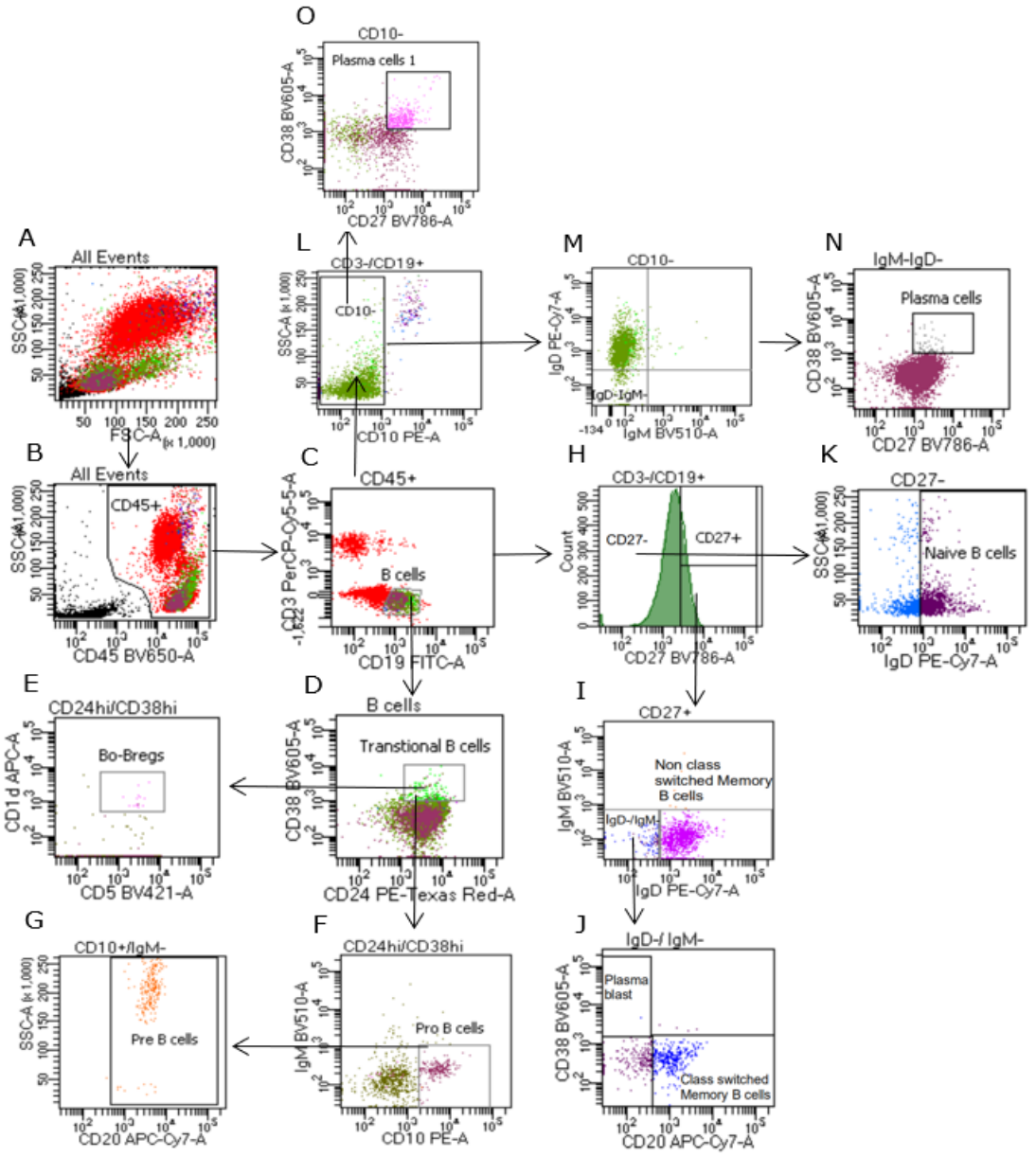

Figure 6. Gating strategies for B cells and their subtypes. (A): All events display; (B): $CD45^+$ leukocytes; (C): $CD3^-/CD19^+$ B lymphocytes; (D): $CD24^{hi}/CD38^{hi}$ transitional B cells; (E): $CD1d^+/CD5^+$ Bregs-1; (F): $CD10^+/IgM^-$ pro B cells; (G): $CD10^+/IgM^-/CD20^+$ pre B cells; (H): $CD27^+$ and $CD27^-$ B cells; (I): IgD^+ n-s memory B cells; (J): $CD20^-/CD38^{hi}$ plasmablasts and $CD20^+/CD38^{+-}$ c-s memory B cells; (K): IgD^+ naive B cells; (L): $CD10^-$ B cells; (M): IgD^-/IgM^-; (N): $CD27^{hi}/CD38^{hi}$ plasma cells; (O): $CD10^-CD27^{hi}/CD38^{hi}$ plasma cells 1.

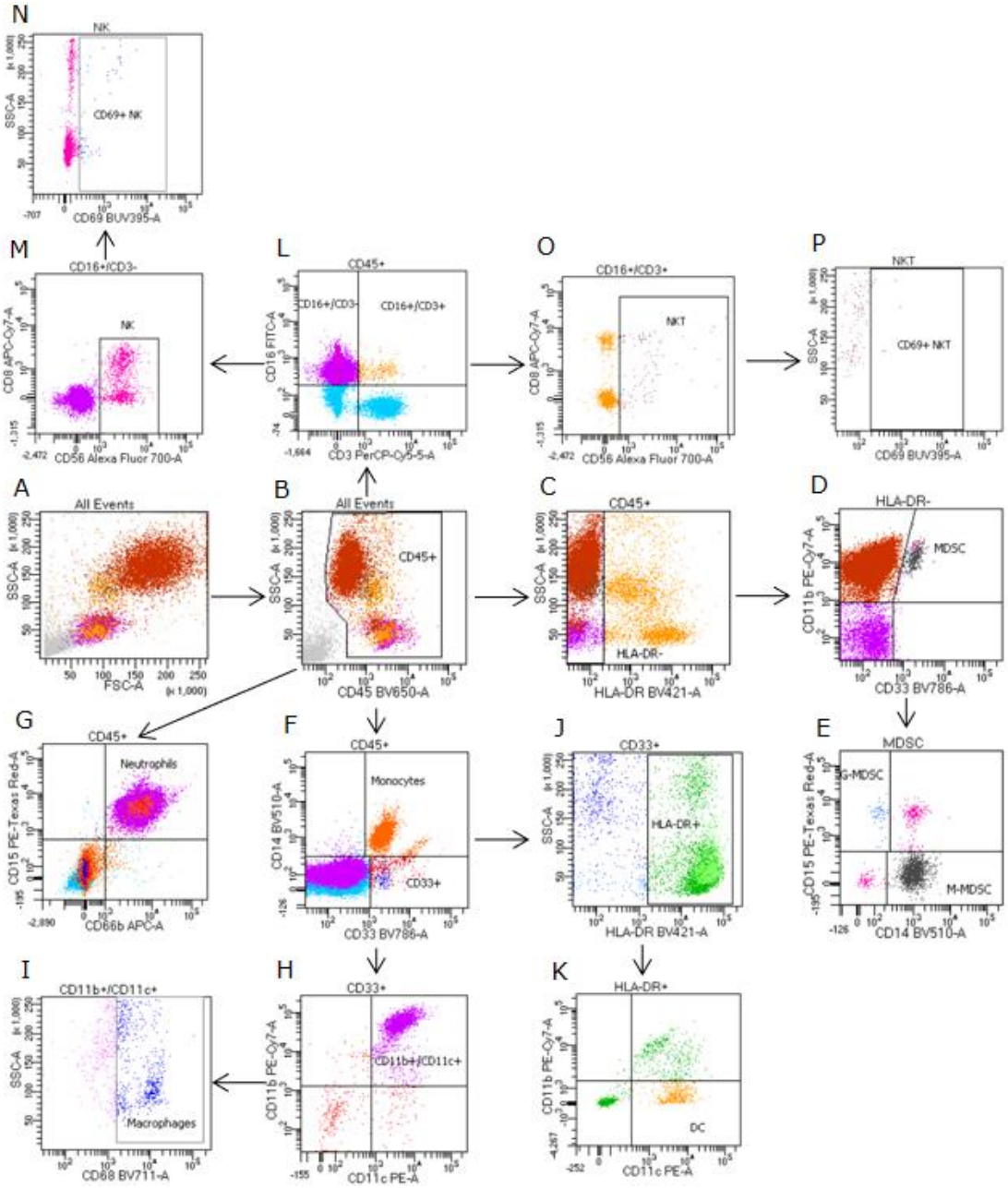

Figure 7. Gating strategies for neutrophils, monocytes, macrophages, DC, NK cells, NKT cells, MDSC, and their subtypes. (A): All events display; (B): CD45+ leukocytes; (C): HLA-DR-; (D): CD11b+/CD33+ MDSC; (E): CD15+/CD14- G-MDSC and CD15-/CD14+ M-MDSC; (F and G): CD15+/CD66b+ neutrophils and DC14+/CD33+ monocytes; (H): $CD33^{+}$; (I): $CD68^{+}$ macrophages; (J): $HLA\text{-}DR^{+}$; (K): $CD11b^{-}/CD11c^{+}$ DC; (L): $CD16^{+}/CD3^{-}$ and $CD16^{+}/CD3^{+}$; (M): $CD56^{+}/CD8^{+-}$ NK; (N): $CD69^{+}$ NK; (O): $CD56^{+}/CD8^{+-}$ NKT; (P): $CD69^{+}$ NKT.

3. Results

3.1. Demographic and Clinicopathological Analysis of Study Subjects

The study cohort comprised fifteen HCC patients who underwent LR. Table 3 shows the demographic characteristics. More than half (60.00%) were male, and the median age of all patients was 71.00 years. We did not process specimens with viral hepatitis, given the impact of hepatitis infection on the immune system and the fact that we considered worker safety. Only 2 (13.00%) patients in the study cohort had alcoholic hepatitis. A small number (3 cases, 20.00%) suffered from cirrhosis. None of the patients suffered from ascites. The liver reserve function of patients was generally well, and only 3 (20.00%) patients had Child-Pugh A cirrhosis, and the rest had no cirrhosis. Preoperative imaging examinations and postoperative pathological reports showed that 13 cases (93.00%) were single tumor lesions. 9 cases (60.00%) and 10 cases (71.00%) were within the criteria of MC and UCSF criteria, respectively. 5 cases (33.00%) had a microvascular invasion. Six cases (40.00%) met the MC pathologically. The UICC staging criteria have four stages. Seven cases（50.00%) were stage 1, 4 cases (29.00%) were stage 2, 1 case (7.00%) was stage 3, and 2 cases (14.00%) were stage 4. The WHO grading criteria include two grades, 1 case (7.00%) was grade A, and 13 cases (93.00%) were grade B. The BCLC grouping criteria included groups A (10 cases, 67.00%) and B (5 cases, 33.00%). The median follow-up time was 21.4 months. A total of nine patients experienced recurrences during the follow-up period. Detailed information about the demographic data can be found in Table 3.

Table 3. Demographics of the study population. Abbreviations: IQR: Interquartile Range; HBV: Hepatitis B; HCV: Hepatitis C; UCSF: University of California at San Francisco; UICC: Union for International Cancer Control; WHO: World Health Organization; BCLC: Barcelona

Clinic Liver Cancer; AFP: Alpha-fetoprotein; ALT: Alanine transaminase; AST: Aspartate transaminase; APTT: Activated partial thromboplastin time; INR: International normalized ratio; CRP: C-reactive protein.

Variables	Results
Gender (Female/Male)	6 (40.0%)/9 (60.0%)
Age (Years) (Median (IQR))	71.00 (12.00)
Ascites (Yes/No)	0 (0.0%)/15 (100.0%)
Cirrhosis (Yes/No)	3 (20.0%)/12 (80.0%)
Child-Pugh grading (0/A)	12 (80.0%)/3 (20.0%)
Hepatitis (HBV/HCV/Alcoholic)	0 (0.0%)/0 (0.0%)/2 (13.0%)
Number of lesions (N=1/N=2)	13 (93.0%)/1 (7.0%)
Milan criteria (imaging) (Inside/Outside)	9 (60.0%)/6 (40.0%)
UCSF imaging (Inside/Outside)	10 (71.0%)/4 (29.0%)
Microvascular invasion (Yes/No)	5 (33.0%)/10 (67.0%)
Milan pathology (Inside/Outside)	6 (40.0%)/9 (60.0%)
UICC staging (1/2/> 2)	7(50.0%)/4 (29.0%)/3 (21.0%)
WHO grading (1/2/3)	1 (7.0%)/13 (93.0%) 0 (0.0%)
BCLC grouping (A/B)	10 (67.0%)/5 (33.0%)
Recurrence (Yes/No)	9 (60.0%)/6 (40.0%)
Follow-up time (Months) (Median (IQR))	21.40 (11.70)
Bilirubin (mg/dL) (Median (IQR))	0.80 (0.30)
Albumin (mg/dL) (Median (IQR))	40.00 (3.00)
AFP (ng/mL) (Median (IQR))	9.95 (80.75)
ALT (U/L) (Median (IQR))	49.00 (34.50)
AST (U/L) (Median (IQR))	53.50 (38.50)
APTT (s) (Median (IQR))	25.00 (2.00)
INR (Median (IQR))	1.00 (0.20)
Creatinine (mg/dL) (Median (IQR))	1.10 (0.30)

CRP (mg/L) (Median (IQR))	2.00 (4.55)
Leukocytes (10^3/µL) (Median (IQR))	6190.00 (2990.00)
Platelets (10^3/µL) (Median (IQR))	198.00 (123.00)

3.2. Correlation Analysis Between Number of Immune Cells and Clinical Characteristics Before Resection

We performed a correlation analysis between immune cells and clinical features. All results can be observed in Supplementary Tables 5 and 6. In the following, I will present only the relevant and significant results. There were statistically significant correlations between the following clinical parameters and preoperative immune cell levels: Patient age was significantly negative correlated with eTh cell counts ($r = -0.833$, $p < 0.001$), and Th1/Th2 ratios ($r = -0.833$, $p < 0.001$). ALT levels were significantly positive correlated with plasmablast counts ($r = 0.788$, $p < 0.001$). INR levels were significantly positive correlated with frequency of MDSC ($r = 0.843$, $p < 0.001$) (Figure 8).

Apart from these singular correlations no clinical parameters, which correlated significantly with the measured preoperative values could be identified.

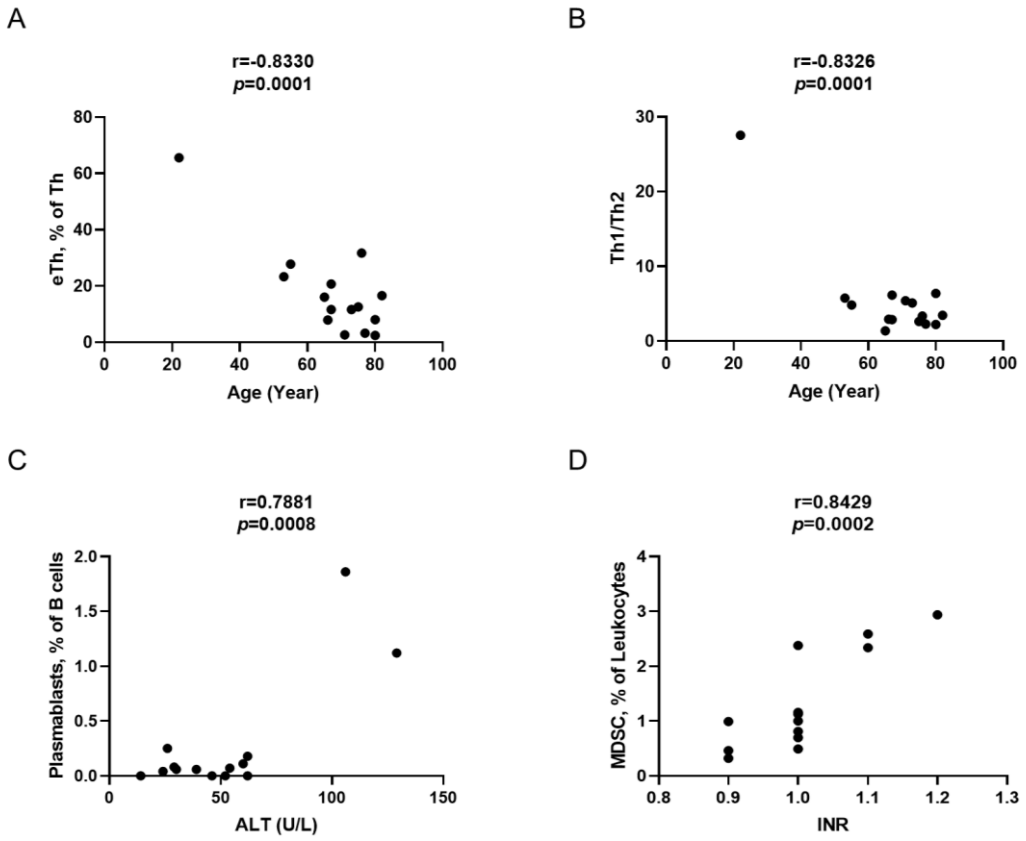

Figure 8. Correlation between the number of immune cells and clinical characteristics prior to resection. (A: Correlation between Age and eTh; B: Correlation between Age and Th1/Th2; C: Correlation between ALT and Plasmablasts; D: Correlation between INR and MDSC; Pearson's correlation coefficient).

3.3. Longitudinal Changes in Distribution of Immune Cells

Statistical calculations were performed 49 different variables at different time points. Most longitudinal changes in immune cell distributions were not significant. Only the frequencies of G-MDSC among MDSC ($p < 0.001$) and M-MDSC among MDSC ($p < 0.001$) showed themselves to be changing significantly throughout the observation period. Whereas G-MDSC increased, M-MDSC fell significantly until POM 12 (Table

4). All other non-significant results can be viewed in Supplementary Table 7.

Table 4. Results with statistical significance following multiple testing corrections.

Cell Type	PreOP (Mean±SD, N=15)	POM 3 (Mean±SD, N=15)	POM 12 (Mean±SD, N=15)	p value
G-MDSC, % of MDSC	18.91±18.64	24.14±21.08	49.09±29.93	**<0.001**
M-MDSC, % of MDSC	57.16±25.84	46.56±34.35	22.36±26.01	**<0.001**

3.4. Multiple Comparison Statistical Calculations for all Detected Subsets at Different Time Points

Furthermore, as described above we compared the differences between all subsets of immune-cells at all time-points. The data showed that compared with PreOP, the proportion of M-MDSC among MDSC was significantly reduced at POM 12 ($p < 0.001$; Figure 9). All other comparisons yielded no significant results that reached the high threshold for significance defined by the Bonferroni correction to protect from multiple testing. All results are shown in Supplementary Table 8.

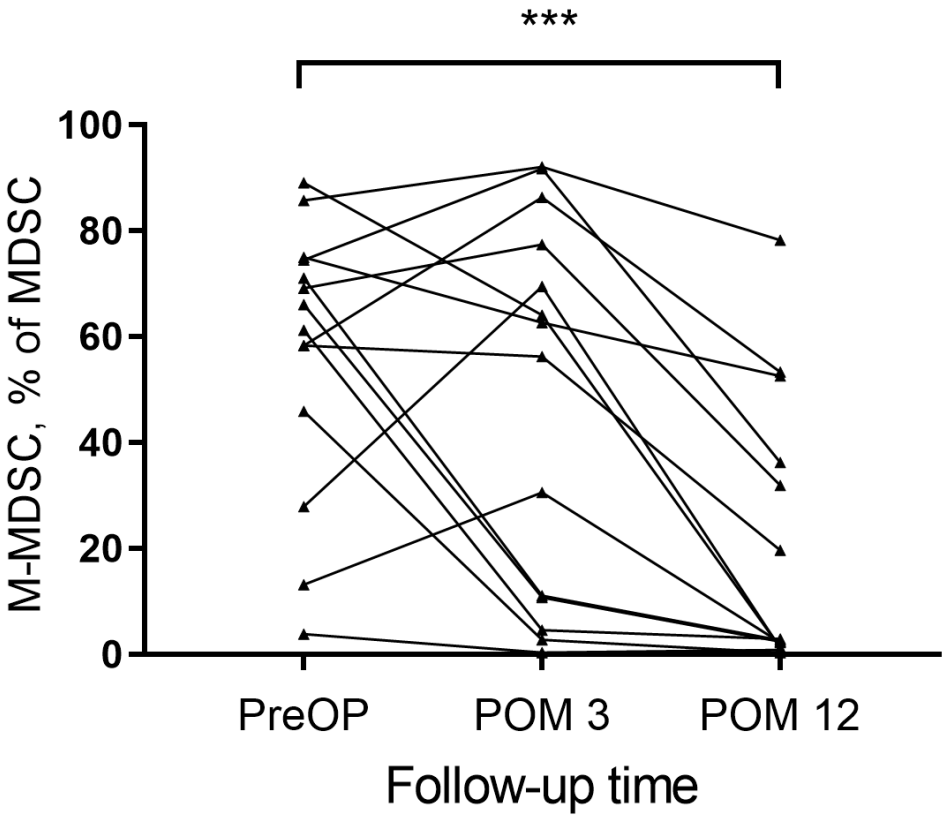

Figure 9. Changes in immune cells during the perioperative period. Abbreviations: PreOP: Pre-operation; POM 3: Postoperative month 3; POM 12: Postoperative month 12. The proportion of M-MDSC among MDSC; one-way repeated measures ANOVA. ***: $p < 0.001$).

3.5. Longitudinal Changes of Immune Cell Distribution Based on Tumor Recurrence

In order to explore the differences in immune cells between patients with recurrence and non-recurrence groups at different time points, patients were divided into recurrence and non-recurrence according to the follow-up. After statistical analysis, we found no statistically significant results in terms of recurrence of HCC. All results acquired by this comparison are listed in Supplementary Table 9.

4. Discussion

The here presented study evaluated the immunological characteristics of the PB of HCC patients over a year-long period to try to accurately describe the long-term immune statuses of patients following surgical resection. This is the first comprehensive assessment of the perioperative immune characteristics of non-HBV/HCV HCC patients who undergo hepatectomies. In the discussion, the relevant results will be described and put into context with the literature.

4.1. Correlation Between Preoperative Immune Cells and Demographic and Clinicopathological Variables

Immune cell distribution was evaluated to whether it correlates with preoperative demographic and clinicopathological variables.

In my results, I could show that the patient age and the count of eTh and Th1/Th2 ratio had a negative correlation. Obviously, there are some studies that have shown that the incidence and prognosis of HCC correlate with age.[302] In fact, some guidelines even propose that people of age should be screened against HCC.[303] However, there are no studies that have reported the correlation between eTh and Th1/Th2 ratio and age in HCC. In general, I could not find any studies describing whether the eTh count or the ratio of Th1 and Th2 is influenced by age at all. It however shows that age might play an important role in the interpretation of Immune cell counts. This is relevant since age always should be regarded as a confounding factor when analysing immune cell distributions.[302]

ALT levels are indicative of liver parenchymal damage. Higher transaminase can indirectly reflect poor liver function but are no good indicator of cirrhosis.[304] Therefore,

our results are mixed. I could show that plasmablast levels were positively correlated with ALT levels. This significant correlation did withstand the low alpha threshold and could uphold its significance. There is limited literature regarding B-cells and their role in liver injury and cirrhosis. Plasmablasts are the short-lived effector cells of the early antibody response and seem to play a role in the immunological response to alcoholic liver disease.[305] This confirms our results in the broadest terms and should be further investigated especially regarding the prognosis of HCC.

In addition, my results showed a significant positive correlation between preoperative INR levels and the frequency of MDSC in HCC. MDSC is a heterogeneous cell population that cells proliferate and activate under pathological conditions (such as tumors). These cells have an immunosuppressive ability and are directly related to tumor immune escape. G-MDSC and M-MDSC are the main subsets of MDSC, both possessing immunosuppressive functions as mentioned above. According to Khaled et al., the frequency of different MDSC subsets appears to relate to different types of cancer. Kidney cancer, colon cancer, and lung cancer showed elevated PB G-MDSC levels, while prostate cancer, HCC, and head and neck cancer demonstrated elevated M-MDSC levels.[194] Chronic hepatitis infection can also affect the immune status of HCC patients. Fang et al. found that the M-MDSC levels of patients with chronic HBV infections were significantly increased, potentially caused by the HBV surface antigen promoting the differentiation of M-MDSC.[306] However, Pallett et al. showed that G-MDSC levels were significantly increased in chronic HBV patients.[307] Gao et al. found that high PB levels of M-MDSC in HCC patients were related to the early recurrence and prognosis following surgery.[192] A similar publication to the in here presented study could find a correlation between MDSC and tumor volume (cm^3).[308] Lee et al. investigated 19 hepatitis B-related HCC patients at 3 time-points related to tumor resection (PreOP, first postoperative week, and first POM).[308] This study did not correlate MDSC with INR, which makes my study the first one to report a correlation

between MDSC and INR in non-HBV/non-HCV patients. However, due to the limitations of the sample size in this study, the characteristics of this correlation between preoperative INR levels and the frequency of MDSC need to be further investigated.

As can be seen, many comparisons and correlations did not reach the high threshold for significance set up by the correction for multiple testing. Based on the literature search and review many potential associations were identified, which however could not be confirmed by our exploratory results.

A tumor-specific CTL response can prolong DFS after HCC treatment.[309] In our results, however, we could not demonstrate an increase in CTL. Furthermore, Wang et al. reported that there is a correlation between MDSC and different Child-Pugh grades. The higher the Child-Pugh grade, the higher the MDSC level. Besides it is hypothesized, that the MDSC ratio and Child-Pugh grade are related to the prognosis of HCC by determining recurrence.[310] My results could not demonstrate this association between the MDSC ratio and the Child-Pugh grade. However, in our patient cohort, only Child-Pugh grade 0 and A patients were operated on, which makes an investigation of an increase of MDSC in this cohort difficult. However, rarely Child-Pugh grade B patients are suitable for resection and therefore we had no patients with this grade within our study cohort.

For years the number and size of tumors have been used to describe unfavorable tumor biology.[148] This is in part reflected by the negative outcomes in patients with a multitude of tumors that are resected.[311] Moreover, it might not only give an insight into tumor biology but also tumor immunology. A weakened immune system allows the HCC nodules to propagate.[188] We hypothesized that in this case that the proportion of nTh would most likely increase in relation due to the consumption of effector Th cells.

However, we were unable to demonstrate this relation. We were also unable to demonstrate a relation between HCC immune cells and the UCSF imaging before surgery. With a higher amount of patients, a clearer picture could emerge, especially regarding the distribution of the subsets of Th cells in patients who are scheduled for HCC resection.

4.2. The Longitudinal Distribution Pattern of Immune Cells

No previous studies have reported on perioperative changes in M-MDSC. However, there is an aforementioned study that investigated the changes in MDSC perioperatively.[308] The results of my research suggest that, compared with PreOP, the frequency of M-MDSC until POM 12 is significantly reduced. One explanation for this may be the surgical removal of tumor lesions, that causes immunosuppression. The removal results in a gradual decrease in M-MDSC levels.[312] The study by Lee et al. could also show a decrease in MDSC already at POM1. Conversely to the trend observed in M-MDSC levels, the current study found that, compared to PreOP and POM 3, G-MDSC levels increased until POM 12. One possible explanation for this is that the frequency of different MDSC subtypes is also related to the type of cancer mentioned above. For example, colon cancer patients have mainly increased G-MDSC in PB, while HCC patients mainly showed elevation of M-MDSC.[194, 313] Within my small sample size I could not demonstrate a significant change in all MDSC perioperatively.

4.3. The Pattern of Longitudinal Distribution of Immune Cells During Tumor Recurrence

Surgical resection is the primary treatment choice for HCC patients with good liver function; however, recurrence remains common. As previously described, common factors that impact recurrence and survival after surgery include patient-specific

variables, such as patient age, gender, active hepatitis infection, cirrhosis, AFP levels, alkaline phosphatase levels, albumin levels, operation time, and perioperative blood transfusion.[314] There are also tumor-specific variables that can impact both, such as the number of tumors, the presence of nodules or vascular invasion, the specific tumor microenvironment, tumor differentiation, and the tumor capsule.[315]

Tumor biological prognostic parameters can aid in describing individual tumor biological characteristics. Of course, all these factors are able to predict tumor recurrence, especially when using modern predictive algorithms such as machine learning. However, no relevant literature exists that reports perioperative biological prognostic parameters that might be influenced by the surgery itself of tumor recurrence and non-recurrence in HCC patients. This is the first follow-up study on the perioperative changes of various immune cells, which tries to understand the immunophenotype pre- and post-surgery of HCC and identify the immunological profile. Related research reported changes in the body's immune cells within a few days after the operation of the digestive system tumor.[171, 235, 237, 241, 247] Only two articles within the literature review reported changes in PB immune cells during the perioperative period in HCC. Chen and colleagues did not investigate the role of Tregs and Bregs in the context of recurrence.[247] Peng et al. conducted a retrospective analysis on the changes in NLR perioperatively and were able to show an association between NLR dynamic and survival.[171] The study of Lee et al. which was published in 2019 and therefore was not part of the initial review is similarly underpowered and was also not able to show the relation between MDSC and recurrence. Associations were only indirect through correlation with tumor-related factors such as AFP and tumor volume (as mentioned above).[308]

Unfortunately, in this pilot study, no statistically significant results in terms of recurrence of HCC could be detected. We hypothesized that non-recurrence patients would have

higher levels of Th2, NKT, and eCTL cells at all three-time points since NKT and eCTL kill tumor cells. In our results, we could not prove a difference in those specific groups.

Similarly, we expected emCTL and cmCTL to be elevated in recurrence patients. These cells show function after re-contact with the antigen[316]. Under the stimulation of antigen, cmCTL cells can rapidly proliferate and differentiate into effector cells. With the small sample size and the high threshold for significance, no difference was measured.

Research on macrophages has uncovered that activated macrophages are divided into classically activated macrophages (M1) and alternately activated macrophages (M2).[317] The M1 subtype functions by phagocytosing tumor cells and presenting antigens, while the M2 subtype, among TAMs that may have the M2 phenotype, is involved in promoting tumor growth, angiogenesis, recurrence, and metastasis.[318] My results showed no difference in this cell group.

4.4. Study Results in the Context of Immunotherapy

The immune system plays a dual role in tumor development. It can destroy tumor cells and inhibit tumor growth. In addition, tumor development can also be promoted by creating conditions in the tumor microenvironment that are conducive to tumor growth and metastasis.[319] The body can recognize and kill tumor cells through T-cell-dominated cellular immunity.[320] Meanwhile, tumor cells secrete cytokines and chemokines to recruit immunosuppressive cells, thereby generating an immunosuppressive tumor microenvironment. These cells with immunosuppressive functions directly inhibit the cytotoxic function of CTL and NK cells by expressing and producing various factors, which suppress the anti-tumor immune response and eventually lead to tumor escape.[321] In the unique immunosuppressive

microenvironment of liver tumors, a large number of immunosuppressive cells are present as mentioned above. Immunosuppressive cells, such as MDSC, Tregs, and TAMs, are thought to be key factors that assist in a tumor's immune surveillance evasion.[322, 323] Immune escape runs through the entire process of the occurrence and development of HCC. Even if surgical resection is performed, recurrence is very common and is the main reason for treatment failure. Therefore, understanding how to minimize postoperative immune escape and stimulate the immune cells to exert maximum tumor-killing ability is a major challenge.

With the intensive research on HCC, immunotherapy has become a novel therapeutic option for HCC. PD-1, PD-L1, or CTLA-4, which are involved in regulating the degree of immune activation, are expressed on immune cells and are the most widely used targets for ICI.[324] Abnormal expression or function of immune checkpoint molecules is associated with the occurrence of many tumors including HCC.[325, 326] ICI can raise the anti-tumor immune response by blocking the inhibitory effect of tumor cells on immune cells that express immune checkpoints or by restoring or enhancing the tumor-killing effects of immune cells. [327] The success seen with this treatment is expected to be extended to other immunotherapy modalities. With continued exploration and research, immunotherapy is likely to become a future pillar of early HCC treatment. As shown in this study parts of the immune response are significantly altered after LR. These changes might be relevant for the potential use of ICI as an adjuvant treatment after LR. Ideally, ICI should be used before early recurrence can occur but should not be wasted during a state of immunosuppression because of the operation itself. The determination of the ideal time points remains to be evaluated in future research. Possibly a measurement of possibly relevant (albeit in this study not significant) cell groups such as NKT, Th2, eCTL, M-MDSC, G-MDSC, and macrophages could help to determine the urgency of adjuvant ICI treatment.

4.5. Research Limitations

There are several limitations in the present study to note. First, the incidence of primary HCC in Germany is low, and few patients met the inclusion criteria for the study. However, although the sample size was limited, we tried to measure the unique immune patterns of the patients at different time points. Because this is a preliminary study it was underpowered. However, in conjunction with the literature, these results might give an input where differences based on the immune cells between recurrence and non-recurrence patients might be found. This study focused on non-HBV/HCV patients, as the inflammatory state caused by hepatitis could have influenced the experimental results. Additionally, due to worker safety considerations, HBV/HCV material cannot be handled within our lab. Therefore, all HBV/HCV patients were excluded. During the experiment, FCM was used to measure the phenotype of PB immune cells. FCM can efficiently and accurately detect immune cell subpopulations; however, some subjectivity in gate settings exists, which may have impacted experimental results and differences with the literature. We implemented quality measures such as standardized training regarding basic knowledge of gate setting, strict gate control strategy, and finally double-checking by a second lab worker which helped ensure the validity and authenticity of our experimental results. Finally, in the systematic review section, following the initial set systematic search strategy, only 2 publications were relevant to my thesis. Moreover, the number of cell types studied is rare, and the postoperative observation and follow-up time is short, making comparison and analysis impossible. Based on this, I expanded my search to include tumors of the entire digestive system. However, since digestive tumors are diverse, this may have lead to conflicting results.

Regarding the study design, we concentrated on 3 different time-points: PreOP, POM3, and POM12. Other studies investigated time-points closer to the operation. We

consciously decided to include a longer time-frame of 12 months. Follow-up conducted at POM 12 can fully reflect the persistent state of the patient's immune system. In fact, at 12 months the immune reaction caused by the operation, which might supersede the immune reaction caused by recurrence or a residual liver lesion is no longer present. Therefore, we believe results from this time point are especially relevant. Since most early recurrence occurs within 24 months this holds the potential to identify recurrence quicker by reducing follow-up periods in risk groups.

5. Conclusion

The goal of this research was to explore the correlation between the clinical characteristics of patients with non-HBV/HCV HCC and PB immune cells, the longitudinal changes in the distribution of immune cells at three different time points during the perioperative period, as well as the immune pattern between postoperative HCC recurrence and non-recurrence patients. It is concluded that a correlation between preoperative peripheral circulating immune cells and clinical parameter variables exists. Furthermore, through the observation period changes in immune cell distribution occurred. It is unlikely that these changes are derived from the trauma of the operation. This study opens the avenue for new research into the immune response against HCC not only perioperatively as a predictive tool but also as a therapeutic target for adjuvant treatment.

6. Summary

Hepatocellular carcinoma (HCC) is a common malignant tumor with a low 5-year survival rate. Surgery is the most common and effective treatment; however, the rate of recurrence is high and the long-term prognosis is poor. LT achieves the best results; however, due to strict inclusion criteria and a lack of transplantable organs, many patients who could have received promising results with LT are excluded. Therefore, LR remains the primary surgical treatment for HCC. Many studies have shown that the occurrence, development, and prognosis of most malignancies, including HCC, are closely related to the immune status of patients. This has been used as a therapeutic target. In recent years, immunotherapy has become an important means of treating cancer. Immune checkpoint inhibitor (ICI) has been a breakthrough for HCC treatment and is likely to be a promising adjuvant treatment option following HCC resection. To fully understand the perioperative immune status of HCC patients, this study undertook a comprehensive assessment of the distribution of peripheral blood (PB) immune cells in non-Hepatitis B/C virus (non-HBV/HCV) HCC patients before resection, 3 months after surgery, and 1 year after the operation.

The preliminary work involved the systematic evaluation of the perioperative changes in PB immune cells of patients with digestive system tumors. Only two studies focused on the changes in PB immune cells in HCC patients during the perioperative period. However, both studies only focused on less cell type at once, included mainly hepatitis patients primarily, and used short postoperative observation and follow-up times. Therefore, a comprehensive comparison and analysis of the distribution of immune cells during the perioperative period was unachievable. This research comprehensively evaluated the immunological characteristics of the PB of HCC patients, conducting a follow-up one-year post-surgery to ascertain the long-term

immune status of patients after resection. Additionally, I compared the immune patterns of recurrence and non-recurrence groups after resection.

My results show that there is a certain correlation between preoperative circulating immune cells and clinical parameters. Compared with pre-operation (PreOP) and post-operation month (POM) 3, the frequency of granulocyte myeloid-derived suppressor cells (G-MDSC) increased significantly one year after resection, while the frequency of mononuclear myeloid-derived suppressor cells (M-MDSC) in POM 12 decreased. Unfortunately, in this preliminary study, no statistically significant results regarding HCC recurrence were found.

In conclusion, through the flow cytometry (FCM) analysis method, we revealed the distribution characteristics of PB immune cells of non-HBV/HCV HCC during perioperative. We found a correlation between preoperative peripheral circulating immune cells and clinical parameters. The immune activation axis and immune suppression axes changed during the perioperative period. Besides, we found that the immune-activated cell groups were possibly more frequent in the postoperative non-recurrence group than in the recurrence group. This study demonstrates that changes in the immune system during the perioperative period can affect the prognosis of HCC and might guide the adjuvant treatment of patients with ICI. It can be used as a predictive tool for the perioperative period. Besides, it can provide a theoretical basis for further research on anti-HCC tumor immune response.

7. Zusammenfassung

Das hepatozelluläre Karzinom (HCC) ist ein häufiger bösartiger Tumor mit einer niedrigen 5-Jahres-Überlebensrate. Die Chirurgie ist die häufigste und wirksamste Behandlungsmethode, allerdings ist die Rückfallquote hoch und die Langzeitprognose schlecht. Die besten Ergebnisse werden mit der LT erzielt. Aufgrund strenger Einschlusskriterien und eines Mangels an transplantierbaren Organen werden jedoch viele Patienten, bei denen die LT vielversprechende Ergebnisse hätte erzielen können, ausgeschlossen. Daher bleibt die LR die primäre chirurgische Behandlung des HCC. Viele Studien haben gezeigt, dass das Auftreten, die Entwicklung und die Prognose der meisten bösartigen Erkrankungen, einschließlich HCC, eng mit dem Immunstatus der Patienten zusammenhängen. Dies wurde als therapeutisches Ziel genutzt. In den letzten Jahren hat sich die Immuntherapie zu einem wichtigen Mittel der Krebsbehandlung entwickelt. Der Immun-Checkpoint-Inhibitor (ICI) war ein Durchbruch für die HCC-Behandlung und ist wahrscheinlich eine vielversprechende adjuvante Behandlungsoption nach HCC-Resektion. Um den perioperativen Immunstatus von HCC-Patienten vollständig zu verstehen, wurde in dieser Studie eine umfassende Bewertung der Verteilung der Immunzellen im peripheren Blut (PB) von HCC-Patienten ohne Hepatitis-B/C-Virus (Nicht-HBV/HCV) vor der Resektion, drei Monate nach der Operation und ein Jahr nach dem Eingriff vorgenommen.

Die Vorarbeiten umfassten die systematische Bewertung der perioperativen Veränderungen in den PB-Immunzellen von Patienten mit Tumoren des Verdauungssystems. Nur zwei Studien befassten sich mit den Veränderungen der PB-Immunzellen bei HCC-Patienten während des perioperativen Zeitraums. Beide Studien konzentrierten sich jedoch nur auf wenige Zelltypen gleichzeitig, schlossen hauptsächlich Hepatitis-Patienten ein und verwendeten kurze postoperative

Beobachtungs- und Nachbeobachtungszeiten. Daher war ein umfassender Vergleich und eine Analyse der Verteilung von Immunzellen während des perioperativen Zeitraums nicht möglich. In dieser Studie wurden die immunologischen Eigenschaften des PB von HCC-Patienten umfassend untersucht und eine Nachuntersuchung ein Jahr nach der Operation durchgeführt, um den langfristigen Immunstatus der Patienten nach der Resektion zu ermitteln. Darüber hinaus verglich ich die Immunmuster der Rezidiv- und Nichtrezidivgruppen nach der Resektion.

Meine Ergebnisse zeigen, dass ein gewisser Zusammenhang zwischen präoperativen zirkulierenden Immunzellen und klinischen Parametern besteht. Im Vergleich zum präoperativen (PreOP) und postoperativen Monat (POM) 3 stieg die Häufigkeit der granulozytären myeloischen Suppressorzellen (G-MDSC) ein Jahr nach der Resektion signifikant an, während die Häufigkeit der mononukleären myeloischen Suppressorzellen (M-MDSC) im POM 12 abnahm. Leider wurden in dieser vorläufigen Studie keine statistisch signifikanten Ergebnisse hinsichtlich des Wiederauftretens von HCC gefunden.

Zusammenfassend lässt sich sagen, dass wir mit Hilfe der durchflusszytometrischen (FCM) Analysemethode die Verteilungseigenschaften der PB-Immunzellen von Nicht-HBV/HCV-HCC während der perioperativen Phase aufdeckten. Wir fanden eine Korrelation zwischen präoperativen peripheren zirkulierenden Immunzellen und klinischen Parametern. Die Achse der Immunaktivierung und die Achse der Immunsuppression veränderten sich während der perioperativen Phase. Außerdem stellten wir fest, dass die immunaktivierten Zellgruppen in der postoperativen Nicht-Rezidiv-Gruppe möglicherweise häufiger waren als in der Rezidiv-Gruppe. Diese Studie zeigt, dass Veränderungen im Immunsystem während des perioperativen Zeitraums die Prognose von HCC beeinflussen können und die adjuvante Behandlung von Patienten mit ICI leiten könnten. Sie kann als prädiktives Instrument für den

perioperativen Zeitraum verwendet werden. Außerdem kann sie eine theoretische Grundlage für die weitere Erforschung der Anti-HCC-Tumor-Immunantwort bilden.

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Supplementary Table 1. Summary of included studies. Abbreviations: HCC: Hepatocellular carcinoma; CRC: Colorectal cancer; Bregs: Regulatory B cells; Tregs: Regulatory T cells; Th: Helper T cells; WBC: White blood cell; NK: Natural killer; NKT: Natural Killer T; NLR: Neutrophil to lymphocyte ratio; CTL: Cytotoxic T lymphocytes; cDC1: Circulating myeloid dendritic cells 1; cDC2: Circulating lymphoid dendritic cells 2; N/A: Data not found; N/M: No experimental methods; N.S.: Data found but have no significance; OS: Overall survival; RFS: Recurrence-free survival; CSS: Cancer-specific survival; DFS: Disease-free survival; PB: Peripheral blood; PBMC: Peripheral blood mononuclear cells; FCA: Flow cytometry analysis; FACS: Fluorescence-activated cell sorting; PreOP: Pre-operation; POD: Postoperative day; POW: Postoperative week; POM: Postoperative month; RAS: Robot-assisted surgery; LS: Laparoscopic surgery; CS: Conventional surgery; VATS: Video-assisted thoracoscopic surgery; MIS: Minimally invasive surgery.

Reference	**Study Population**				**Study Cell Type**				**Follow-up measurement time-points**	**Changing tendency**	**Survival**
	Patients	Amount	Region	Treatment	Cell Marker	Cell Type	Cell Source	Method			
Miyatani, K., et al. 2018[235]	Gastric cancer	280	Japan	CS	N/A	NLR	PB	Cell count	POM 1	POM 1: Both PreOP NLR high and POM 1 NLR high Either PreOP NLR high or	5 years of survival: 58.1%

										POM 1 NLR high Both PreOP NLR low and POM 1 NLR low	75.1% 92.8%
Peng, W., et al. 2014[171]	HCC	189	China	CS	N/A	NLR	N/A	N/M	POM 1	POM 1: Increased group: 80 patients Decreased group: 109 patients	Increased group: Poor OS and RFS than NLR decreased group
Kubo, T., et al. 2014[241]	CRC	524	Japan	CS	N/A	NLR	PB	Cell count	POD 1 and POD 3	Divided patients (include Pre, POD 1, and POD 3) into high NLR group and low NLR group	High perioperative NLR score: worse CSS and DFS
Wang, Y., et	CRC	7	China	CS	N/A	T lymphocyte %	PBMC	FCA	POW 1	POW 1: N.S.	N/A

al. 2017[236]						NK lymphocyte % NKT lymphocyte %					
Tan, JH., et al. 2016[237]	Esophageal cancer	228 Include: VATS: 52 CS: 176	China	CS MIS (VATS)	N/A	$CD3^+$ cells $CD4^+$ cells $CD8^+$ cells $CD4^+/CD8^+$ ratio NK cells	PB	FCA	POD 1 and POD 7	$CD3^+$ cells, $CD4^+$ cells, NK cells and $CD4^+/CD8^+$ ratio: VATS: POD 1: decreased, then return to PreOP level on POD 7 CS: POD 1: decreased, then increased, but POD 7 still lower than PreOP $CD8^+$ T cells: N.S.	N/A
Shibata, J., et al. 2015[238]	CRC	46 Include: RAS:	Japan	CS MIS (RAS)	$CD3^-/CD56^+$ $CD3^+/CD8^+$	NK cells CTL	PB	FCA	POD 1, POD 3, and POD 6	NK cells, CTL and Th: from PreOP to POD 1: decreased, POD 3 and POD 6: increased	N/A

		15 LS: 23; CS: 8			$CD3^+/CD4^+$ $CD3^-/CD19^+$	Th B lymphocytes				B lymphocytes: no significant change	
Ling, L., et al. 2015[239]	CRC	31	China	CS	Th1: IL-17-IL-22-IFN-γ^+CD4^+ Th17: $IL-17^+IL-22-IFN-\gamma-CD4^+$ Th22: IL-17-$IL-22^+IFN-\gamma-CD4^+$	Th1 Th17 Th22 $IL-17^+IL-22^+IFN-\gamma-CD4^+$ T cells	PB	FCA	POD 14	Th1%: POD 14: N.S Th17%, Th22%, and $IL-17^+IL-22^+IFN-\gamma-CD4^+$ T cells%: POD 14 were significantly higher than PreOP	N/A
Fujii, K., et al. 2003[244]	Gastric cancer	20 Include: LS: 10	Japan	CS MIS (LS)	Activated NK cell: $CD57^+$ Activated lymphocyte:	WBC Lymphocytes	PBMC	FCA	POD 1, 3, and POD 7	WBC: increased on POD 1, then return to PreOP level on POD 7 Lymphocyte: decreased on	N/A

		and CS: 10			HLA-DR$^+$	CD3$^+$ CD4$^+$ CD8$^+$ CD57$^+$ HLA-DR$^+$				POD 1, then maintain a low level CD3$^+$, CD4$^+$, CD8$^+$, CD57$^+$ and HLA-DR$^+$: decreased on POD 1, then return to PreOP level on POD 7	
Ordemann, J., et al. 2001[249]	CRC	40 Include LS: 20 and CS: 20	Germany	CS MIS (LS)	N/A	WBC CD4$^+$ lymphocytes CD8$^+$ lymphocytes CD4$^+$/CD8$^+$ ratio	PB	FACS	POD 1, 2, 4, and POD 7	WBC: increased on POD 1, then return to PreOP level on POD 7 CD4$^+$ lymphocytes, CD8$^+$ lymphocytes and CD4$^+$/CD8$^+$ ratio: no significant change after surgery	N/A
Helvind, NM., et al.	Colonic cancer	263	The	MIS (LS	N/A	WBC	N/A	N/M	POD 1, POD 2, and	LS: PreOP to POD 1:	N/A

2013[248]		Include LS: 162 and RAS: 101	Netherlands	and RAS)					POD 3	increased POD 1 to POD 3: decreased RAS: PreOP to POD 2: increased POD 2 to POD 3: decreased	
Tezuka, k., et al. 2012[242]	Pancreatic tumor	53	Japan	CS	N/A	WBC	N/A	N/M	POD 1, 2, 3, 5, 7, POW 2, POM 1 and POM 3	PreOP to POD 2: increased, then decreased until POM 3	N/A
Takaya, S., et al. 2015[246]	Gastric Cancer	33	Japan	CS	N/A	Lymphocytes WBC	PB	FCA	POD 1, 3, 7, and POD 30	Decreased on POD 1 and then increased, return to PreOP level on POD 30 Increased on POD 1, then return to the PreOP level on POD 30	N/A

Maas, K.W., et al. 2014[250]	Esophageal cancer	27 Include CS: 13 and LS: 14	The Netherlands	CS MIS (LS)	N/A	WBC	PB	FCA	POD 1, POD 3, POD 4, and POD 7	Increased on POD 1, then decreased until POD 4. But the CS group increased on POD 7	N/A
Takahashi, K., et al. 2006[243]	Pancreatic cancer	20	Japan	CS	$CD11c^+$ DCs $CD11c^-$ DCs $CD14^-/CD56^+$ $CD3^+/CD4^+$ $CD3^+/CD8^+$	cDC1 cDC2 NK cells $CD4^+$ T lymphocytes $CD8^+$ T lymphocytes cDC1/cDC2 ratio	PB	FCA	POM 12	cDC1 and cDC1/cDC2 ratio increased in POM 12 cDC2: N.S $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and NK cells: no significant change in POM 12	cDC1 count and cDC1/cDC2 ratio normalized in POM 12: no obvious local recurrence or distant metastasis

Leung, K.L., et al. 2003[245]	Rectosigmoid carcinoma	40 Include LS: 20 and CS 20	Hong Kong	CS MIS (LS)	T cell: $CD3^+$ T cell activation: $CD3^+$ $HLA\text{-}Dr^+$ Non-MHC restricted NK cell: $CD3^-$ $CD16^+CD56^+$ MHC-restricted NK-like cell: $CD3^+CD16^+CD56^+$ Helper T cell: $CD3^+CD4^+$ Cytotoxic T cell:	T cells T cell activation Non-MHC restricted NK cells MHC-restricted NK-like cells Helper T cells Cytotoxic T cells NK cells WBC Lymphocytes B cells	PB	Cell count	POD 1, POD 3, and POD 8	WBC: increased on POD 1, then return to PreOP level on POD 8 Lymphocytes, T cells, B cells, Non-MHC restricted NK cells, NK cells, Natural Killer-like T cells, Cytotoxic T cell, Helper T cells, T cell activation: decreased on POD 1, then return to PreOP level on POD 8	N/A

					$CD3^+CD8^+$ NK cell: $CD3^-$ $CD16^+CD56^+$						
Chen, T., et al. 2012[247]	HCC	36	China	CS	$CD4^+CD25^+CD127^-$	Tregs	PBMC	FCA	POD 1 and POD 7	Tregs and Bregs: increased on POD 1, especially on POD 7 Lymphocytes: decreased on POD 1 and return to the PreOP level on POD 7	N/A
					$CD19^+IL\text{-}10^+$	Bregs					
						Lymphocytes					
Shi, J., et al. 2014[240]	Esophageal cancer	60	China	CS	$CD5^+CD19^+$	Bregs	PBMC	FCA	POD 1 and POD 7	From POD 1 to POD 7: decreased	N/A

Supplementary Table 2. Dedicated T cell and its subsets by 5 staining panels. (Tube 1, as a blank control group; Tube 2-4, as FMO control groups; Tube 5, all antibody added). Tube 1, no antibody was added; Tube 2, which involve all the antibodies except antibody CD197, CD25, CD196, and HLA-DR; Tube 3, which consist of all the antibodies apart from antibody CD194 and CD127; Tube 4, which comprises all the antibodies except antibody CD38 and CD45RO; Tube 5, which contain all the antibodies.

Tube	**Antibody**											
Tube 1	--	--	--	--	--	--	--	--	--	--	--	--
Tube 2	CD4	--	CD194	CD38	CD45	--	CD3	--	CD127	CD45RO	--	CD8
Tube 3	CD4	CD197	--	CD38	CD45	CD25	CD3	CD196	--	CD45RO	HLA-DR	CD8
Tube 4	CD4	CD197	CD194	--	CD45	CD25	CD3	CD196	CD127	--	HLA-DR	CD8
Tube 5	CD4	CD197	CD194	CD38	CD45	CD25	CD3	CD196	CD127	CD45RO	HLA-DR	CD8
Amount (μl)	1	1	1	1	3	1	1	1	1	1	5	1

Supplementary Table 3. Dedicated B cell and its subsets by 6 staining panels. (Tube 6, as a blank control group; Tube 7-10, as FMO control groups; *, Intracellular antibody; --, no antibody added). Tube 6, no antibody was added; Tube 7, which involve all the antibodies except antibody CD5, CD10 and CD1d; Tube 8, which consist of all the antibodies apart from antibody IgM, CD24 and CD20; Tube 9, which comprises all the antibodies except antibody CD38 and IgD; Tube 10, which incorporate all the antibodies except antibody CD27; Tube 11, which contain all the antibodies.

Tube	**Antibody**											
Tube 6	--	--	--	--	--	--	--	--	--	--	--	--
Tube 7	--	IgM	CD38	CD45	CD27	CD19	CD3	--	CD24	IgD	--	CD20*
Tube 8	CD5	--	CD38	CD45	CD27	CD19	CD3	CD10	--	IgD	CD1d	--
Tube 9	CD5	IgM	--	CD45	CD27	CD19	CD3	CD10	CD24	--	CD1d	CD20*
Tube 10	CD5	IgM	CD38	CD45	--	CD19	CD3	CD10	CD24	IgD	CD1d	CD20*
Tube 11	CD5	IgM	CD38	CD45	CD27	CD19	CD3	CD10	CD24	IgD	CD1d	CD20*
Amount (μl)	2	5	2	3	2	5	2	5	2	2	2	2

Supplementary Table 4. Dedicated neutrophils, monocytes, macrophages, DC, NK, NKT and MDSC by 6 staining panels. (Tube 12, as a blank control group; Tube 13-16, as FMO control groups; *, Intracellular antibody; --, no antibody added). Tube 12, no antibody was added; Tube 13, which involve all the antibodies except antibody CD69, CD68, CD16, CD11c, and CD66b; Tube 14, which consist of all the antibodies apart from antibody HLA-DR, CD15, and CD56; Tube 15, which comprises all the antibodies except antibody CD14 and CD11b; Tube 16, which incorporate all the antibodies except antibody CD33; Tube 17, which contain all the antibodies.

Tube	**Antibody**													
Tube 12	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Tube 13	--	--	HLA-DR	CD14	CD45	CD33	--	CD3	--	CD15	CD11b	--	CD56	CD8
Tube 14	CD69	CD68*	--	CD14	CD45	CD33	CD16	CD3	CD11c	--	CD11b	CD66b	--	CD8
Tube 15	CD69	CD68*	HLA-DR	--	CD45	CD33	CD16	CD3	CD11c	CD15	--	CD66b	CD56	CD8
Tube 16	CD69	CD68*	HLA-DR	CD14	CD45	--	CD16	CD3	CD11c	CD15	CD11b	CD66b	CD56	CD8
Tube 17	CD69	CD68*	HLA-DR	CD14	CD45	CD33	CD16	CD3	CD11c	CD15	CD11b	CD66b	CD56	CD8
Amount (μl)	5	2	3	4	3	2	4	2	5	2	2	2	5	2

Supplementary Table 5. Correlation between immune cells and demographic parameters. Abbreviations: aCTL: Activated cytotoxic T lymphocytes; aTh: Activated helper T cells; aTregs: Activated Tregs; Bregs: Regulatory B cells; cmCTL: Central memory cytotoxic T cells; cmTh: Central memory helper T cells; CTL: Cytotoxic T lymphocytes; cs-memory B cells: Class-switched memory B cells; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; eTh: Effector helper T cells; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; G-MDSC: Granulocyte-like MDSC; pro B: Progenitor B cells; pre B: Precursor B cells; MDSC: Myeloid-derived suppressor cells; M-MDSC: Monocyte-like MDSC; mTregs: Memory Tregs; maTregs: Memory-activated Tregs; ns-memory B cells: non class-switched memory B cells; nCTL: Naïve cytotoxic T lymphocytes; nTh: Naïve helper T cells; nTregs: Naive Tregs; NK: Natural killer cells; NKT: Natural killer T cells; Tregs: Regulatory T cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells; AFP: Alpha-fetoprotein; ALT: Alanine transaminase; APTT: Activated partial thromboplastin time; AST: Aspartate transaminase; BCLC: Barcelona Clinic Liver Cancer; CRP: C-reactive protein; INR: International normalized ratio; UCSF: University of California at San Francisco; UICC: Union for International Cancer Control. ***: $p < 0.001$.

Clinical Parameters	Gender	UICC Staging	Cirrhosis	Child-Pugh Grading
	p-value	p-value	p-value	p-value
Neutrophils, % of Leukocytes	>0.999	0.448	0.456	0.456
Monocytes, % of Leukocytes	0.852	0.970	0.170	0.170
Macrophages, % of Leukocytes	0.328	0.545	0.316	0.316

DC, % of Leukocytes	0.829	0.083	>0.999	>0.999
MDSC, % of Leukocytes	0.662	0.196	0.126	0.126
G-MDSC, % of MDSC	0.020	0.548	>0.999	>0.999
M-MDSC, % of MDSC	0.043	0.221	0.769	0.769
G-MDSC, % of Leukocytes	0.014	0.351	0.055	0.055
M-MDSC, % of Leukocytes	0.181	0.144	0.456	0.456
NK cells, % of Leukocytes	0.950	0.117	0.456	0.456
NKT cells, % of Leukocytes	>0.999	0.132	0.478	0.478
$CD69^{+}$NK cells, % of NK cells	0.852	0.792	0.456	0.456
$CD69^{+}$NKT cells, % of NKT cells	0.433	0.280	0.349	0.349

B cells, % of Leukocytes	0.191	0.008	0.242	0.242
ns-memory B cells, % of B cells	0.388	0.558	0.945	0.945
Naive B cells, % of B cells	0.529	0.910	0.633	0.633
cs-memory B cells, % of B cells	0.689	0.906	0.365	0.365
Plasma cells, % of B cells	0.881	0.664	0.121	0.121
Plasma cells 1, % of B cells	0.662	0.647	0.368	0.368
Plasmablasts, % of B cells	0.705	0.505	0.160	0.160
Transitional B cells, % of B cells	0.955	0.536	0.734	0.734
Bregs-1, % of Transitional B cells	0.954	0.159	0.539	0.539
Pro B cells, % of Transitional B cells	0.955	0.407	0.295	0.295

Pre B cells, % of Pro B cells	0.345	0.892	>0.999	>0.999
T cells, % of Leukocytes	0.224	0.277	0.734	0.734
Th, % of T cells	0.887	0.925	0.354	0.354
Th17, % of Th	0.088	0.041	0.633	0.633
Th1, % of Th	0.114	0.073	0.945	0.945
Th2, % of Th	0.145	0.143	0.448	0.448
emTh, % of Th	0.066	0.039	0.180	0.180
cmTh, % of Th	0.887	0.177	0.754	0.754
eTh, % of Th	0.607	0.188	0.945	0.945
nTh, % of Th	>0.999	0.078	0.945	0.945

aTh, % of Th	0.388	0.477	0.233	0.233
CTL, % of T cells	0.864	0.978	0.734	0.734
emCTL, % of CTL	0.388	0.534	0.734	0.734
cmCTL, % of CTL	0.529	0.501	0.536	0.536
eCTL, % of CTL	0.607	0.532	0.734	0.734
nCTL, % of CTL	0.145	0.723	0.295	0.295
aCTL, % of CTL	0.689	0.545	0.048	0.048
Tregs, % of Th	0.181	0.366	0.633	0.633
mTregs, % of Tregs	0.607	0.117	0.233	0.233
maTregs, % of Tregs	0.088	0.003	0.945	0.945

nTregs, % of Tregs	0.628	0.538	0.295	0.295
aTregs, % of Tregs	0.190	0.411	0.088	0.088
Th/CTL	0.689	0.357	0.840	0.840
Th1/Th2	0.114	0.088	0.840	0.840
Th1/Th17	0.046	0.039	0.754	0.754
Neutrophils/Lymphocytes	0.228	0.151	>0.999	>0.999

Clinical Parameters	**Recurrence**	**Milan Imaging**	**UCSF Imaging**	**BCLC Grouping**
	p-value	p-value	p-value	p-value
Neutrophils, % of Leukocytes	0.852	0.519	0.692	0.945

Monocytes, % of Leukocytes	0.662	0.364	>0.999	0.945
Macrophages, % of Leukocytes	0.638	0.347	0.549	0.706
DC, % of Leukocytes	0.295	0.674	0.983	0.608
MDSC, % of Leukocytes	0.662	0.438	0.112	0.945
G-MDSC, % of MDSC	0.020	0.606	0.811	0.839
M-MDSC, % of MDSC	0.181	>0.999	0.811	0.945
G-MDSC, % of Leukocytes	0.022	0.252	0.388	>0.999
M-MDSC, % of Leukocytes	0.414	0.606	0.287	0.839
NK cells, % of Leukocytes	>0.999	0.898	0.371	0.188
NKT cells, % of Leukocytes	0.298	0.450	>0.999	0.307

$CD69^{+}$NK cells, % of NK cells	0.142	0.519	0.287	0.304
$CD69^{+}$NKT cells, % of NKT cells	0.151	0.723	0.227	0.070
B cells, % of Leukocytes	0.888	0.474	0.252	0.533
ns-memory B cells, % of B cells	0.456	0.689	0.188	0.440
Naive B cells, % of B cells	0.026	0.456	0.839	0.953
cs-memory B cells, % of B cells	0.328	0.955	0.142	0.310
Plasma cells, % of B cells	0.823	0.457	0.273	0.252
Plasma cells 1, % of B cells	0.950	0.699	0.287	0.733
Plasmablasts, % of B cells	0.885	0.463	0.146	0.653
Transitional B cells, % of B cells	0.776	0.776	>0.999	0.953

Bregs-1, % of Transitional B cells	0.325	0.386	0.301	0.860
Pro B cells, % of Transitional B cells	0.864	0.689	0.945	0.514
Pre B cells, % of Pro B cells	0.181	0.950	0.711	0.190
T cells, % of Leukocytes	0.224	0.864	0.945	0.768
Th, % of T cells	0.285	0.798	0.472	0.456
Th17, % of Th	0.224	0.224	0.188	0.679
Th1, % of Th	0.456	0.328	0.374	0.594
Th2, % of Th	0.607	0.388	0.024	0.768
emTh, % of Th	0.529	0.114	0.008	0.594
cmTh, % of Th	0.313	0.235	0.357	0.613

eTh, % of Th	0.607	0.181	0.540	0.768
nTh, % of Th	0.456	0.036	0.142	0.768
aTh, % of Th	0.529	0.529	0.945	0.440
CTL, % of T cells	0.181	0.776	0.374	0.679
emCTL, % of CTL	0.955	0.456	0.945	0.768
cmCTL, % of CTL	0.955	0.955	0.945	0.165
eCTL, % of CTL	0.955	0.955	0.945	0.310
nCTL, % of CTL	>0.999	0.607	0.304	0.679
aCTL, % of CTL	>0.999	0.607	0.839	0.207
Tregs, % of Th	0.181	0.955	0.454	0.440

mTregs, % of Tregs	0.529	0.328	0.036	0.440
maTregs, % of Tregs	0.607	0.388	0.240	0.953
nTregs, % of Tregs	0.607	0.955	0.454	0.768
aTregs, % of Tregs	0.171	0.981	0.918	0.978
Th/CTL	0.114	0.529	0.454	0.514
Th1/Th2	0.456	0.224	0.076	0.953
Th1/Th17	0.372	0.372	0.357	0.613
Neutrophils/Lymphocytes	0.282	>0.999	>0.999	0.733

Clinical Parameters	Microvascular Invasion	Milan Pathology	Age		Platelets	
	p-value	p-value	r-value	p-value	r-value	p-value
Neutrophils, % of Leukocytes	0.839	0.108	-0.181	0.537	0.088	0.766
Monocytes, % of Leukocytes	>0.999	0.491	0.234	0.422	0.113	0.700
Macrophages, % of Leukocytes	0.390	0.827	0.380	0.181	0.073	0.803
DC, % of Leukocytes	0.051	0.641	0.450	0.106	0.401	0.156
MDSC, % of Leukocytes	0.240	0.142	-0.017	0.953	-0.499	0.070
G-MDSC, % of MDSC	0.374	0.662	0.044	0.882	-0.124	0.674
M-MDSC, % of MDSC	0.635	0.852	-0.154	0.599	-0.072	0.807
G-MDSC, % of Leukocytes	0.390	0.362	-0.045	0.879	-0.220	0.450

M-MDSC, % of Leukocytes	0.304	0.181	-0.065	0.826	-0.467	0.092
NK cells, % of Leukocytes	0.024	0.282	0.090	0.761	0.213	0.466
NKT cells, % of Leukocytes	0.090	0.352	-0.255	0.379	0.309	0.283
$CD69^{+}$NK cells, % of NK cells	0.945	0.852	-0.280	0.332	0.118	0.687
$CD69^{+}$NKT cells, % of NKT cells	0.614	0.550	0.139	0.635	-0.039	0.895
B cells, % of Leukocytes	0.037	0.071	0.186	0.508	0.288	0.297
ns-memory B cells, % of B cells	0.075	0.955	0.259	0.350	0.010	0.972
Naive B cells, % of B cells	0.953	0.864	-0.413	0.126	0.158	0.573
cs-memory B cells, % of B cells	0.371	0.607	-0.012	0.966	0.078	0.783
Plasma cells, % of B cells	0.051	0.077	-0.561	0.037	0.015	0.960

Plasma cells 1, % of B cells	0.036	0.573	-0.424	0.131	-0.008	0.978
Plasmablasts, % of B cells	0.653	0.974	-0.471	0.077	0.240	0.330
Transitional B cells, % of B cells	0.310	0.328	-0.184	0.512	-0.091	0.748
Bregs-1, % of Transitional B cells	0.074	0.325	0.171	0.541	0.630	0.012
Pro B cells, % of Transitional B cells	0.207	0.529	-0.274	0.324	0.526	0.044
Pre B cells, % of Pro B cells	0.438	0.699	-0.116	0.693	0.116	0.694
T cells, % of Leukocytes	0.371	0.529	0.171	0.542	0.014	0.960
Th, % of T cells	0.423	>0.999	0.268	0.334	-0.307	0.266
Th17, % of Th	0.679	0.224	0.474	0.075	-0.347	0.205
Th1, % of Th	0.371	0.328	-0.420	0.119	0.246	0.378

Th2, % of Th	0.859	0.689	0.598	0.019	-0.517	0.049
emTh, % of Th	0.207	0.088	0.304	0.271	-0.413	0.126
cmTh, % of Th	0.195	0.549	0.475	0.073	0.166	0.555
eTh, % of Th	0.594	0.955	-0.833	**<0.001**	0.042	0.882
nTh, % of Th	0.129	0.224	0.363	0.184	0.337	0.219
aTh, % of Th	0.207	0.607	0.447	0.095	-0.293	0.290
CTL, % of T cells	0.679	0.776	-0.224	0.423	0.287	0.301
emCTL, % of CTL	0.129	0.864	0.615	0.015	0.042	0.883
cmCTL, % of CTL	0.679	0.955	0.293	0.289	-0.407	0.132
eCTL, % of CTL	0.953	0.864	-0.584	0.022	0.135	0.631

nCTL, % of CTL	0.679	0.224	0.006	0.984	0.053	0.853
aCTL, % of CTL	0.129	0.776	0.285	0.303	-0.228	0.415
Tregs, % of Th	0.514	0.388	-0.112	0.692	-0.182	0.517
mTregs, % of Tregs	0.440	0.181	0.228	0.414	-0.182	0.515
maTregs, % of Tregs	0.075	0.689	0.522	0.046	0.182	0.517
nTregs, % of Tregs	0.310	0.388	-0.745	0.001	-0.001	0.996
aTregs, % of Tregs	0.071	0.838	-0.260	0.350	-0.577	0.024
Th/CTL	0.859	0.607	-0.132	0.639	0.060	0.834
Th1/Th2	0.953	0.224	-0.833	**<0.001**	0.319	0.246
Th1/Th17	0.456	0.285	-0.715	0.003	0.269	0.332

Neutrophils/Lymphocytes	0.954	0.573	-0.409	0.147	0.196	0.501

Clinical Parameters	**Leukocytes**		**Bilirubin**		**Albumin**		**AFP**	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Neutrophils, % of Leukocytes	0.147	0.264	-0.016	0.956	0.076	0.805	-0.293	0.482
Monocytes, % of Leukocytes	-0.213	0.571	0.354	0.214	0.143	0.641	0.278	0.504
Macrophages, % of Leukocytes	-0.110	0.708	0.009	0.977	0.092	0.764	0.151	0.721
DC, % of Leukocytes	0.124	0.672	0.037	0.900	0.315	0.295	0.481	0.228
MDSC, % of Leukocytes	-0.668	0.009	0.277	0.338	-0.487	0.091	-0.170	0.693
G-MDSC, % of MDSC	-0.220	0.451	-0.063	0.830	-0.189	0.536	0.039	0.923

M-MDSC, % of MDSC	0.265	0.361	-0.141	0.630	0.151	0.623	-0.384	0.348
G-MDSC, % of Leukocytes	-0.552	0.041	0.103	0.727	-0.385	0.194	-0.225	0.592
M-MDSC, % of Leukocytes	-0.418	0.137	0.101	0.731	-0.414	0.160	-0.136	0.749
NK cells, % of Leukocytes	-0.024	0.935	-0.347	0.225	0.050	0.872	0.624	0.098
NKT cells, % of Leukocytes	0.322	0.261	-0.444	0.112	0.422	0.151	0.353	0.392
$CD69^{+}$NK cells, % of NK cells	0.201	0.492	0.038	0.897	0.133	0.665	0.136	0.748
$CD69^{+}$NKT cells, % of NKT cells	-0.119	0.685	-0.084	0.775	-0.215	0.481	0.543	0.164
B cells, % of Leukocytes	-0.093	0.743	-0.121	0.667	0.066	0.821	0.886	0.002
ns-memory B cells, % of B cells	0.442	0.099	0.071	0.802	0.307	0.285	-0.320	0.401
Naive B cells, % of B cells	-0.048	0.866	-0.232	0.406	-0.053	0.857	-0.293	0.445

cs-memory B cells, % of B cells	0.283	0.308	-0.168	0.549	0.286	0.323	-0.302	0.431
Plasma cells, % of B cells	0.038	0.897	0.287	0.320	0.180	0.556	-0.315	0.447
Plasma cells 1, % of B cells	0.038	0.898	0.408	0.148	0.267	0.378	-0.292	0.483
Plasmablasts, % of B cells	-0.146	0.604	-0.087	0.758	0.029	0.922	0.653	0.056
Transitional B cells, % of B cells	-0.515	0.049	0.342	0.212	-0.010	0.974	0.197	0.612
Bregs-1, % of Transitional B cells	0.541	0.038	-0.354	0.195	0.254	0.381	0.296	0.439
Pro B cells, % of Transitional B cells	0.503	0.056	-0.556	0.031	0.149	0.611	-0.003	0.994
Pre B cells, % of Pro B cells	-0.267	0.356	0.197	0.500	0.062	0.840	0.066	0.876
T cells, % of Leukocytes	-0.403	0.137	-0.141	0.617	-0.234	0.420	0.394	0.294
Th, % of T cells	-0.036	0.271	0.335	0.222	-0.061	0.835	-0.094	0.810

Th17, % of Th	-0.305	0.269	0.358	0.190	-0.314	0.275	0.350	0.356
Th1, % of Th	0.304	0.270	-0.158	0.574	0.392	0.165	-0.454	0.220
Th2, % of Th	-0.293	0.290	0.257	0.355	-0.414	0.141	-0.014	0.972
emTh, % of Th	-0.346	0.206	0.039	0.892	-0.394	0.163	-0.288	0.453
cmTh, % of Th	-0.125	0.658	0.343	0.211	0.231	0.428	0.806	0.009
eTh, % of Th	0.152	0.589	-0.267	0.335	0.002	0.995	-0.448	0.227
nTh, % of Th	0.337	0.219	0.037	0.895	0.313	0.276	0.749	0.020
aTh, % of Th	-0.052	0.855	-0.010	0.972	-0.319	0.266	0.112	0.774
CTL, % of T cells	0.281	0.311	-0.248	0.372	0.058	0.844	0.115	0.768
emCTL, % of CTL	-0.365	0.181	0.135	0.632	-0.073	0.803	0.492	0.179

cmCTL, % of CTL	-0.331	0.229	0.448	0.094	-0.104	0.723	-0.200	0.605
eCTL, % of CTL	0.375	0.168	-0.314	0.254	0.023	0.939	-0.272	0.481
nCTL, % of CTL	0.178	0.526	0.089	0.751	0.359	0.207	0.049	0.900
aCTL, % of CTL	-0.060	0.833	0.163	0.561	0.033	0.910	-0.279	0.467
Tregs, % of Th	0.086	0.760	0.031	0.914	-0.142	0.628	-0.145	0.711
mTregs, % of Tregs	-0.124	0.661	-0.009	0.976	0.214	0.462	-0.344	0.365
maTregs, % of Tregs	-0.024	0.933	0.052	0.855	-0.183	0.530	0.802	0.009
nTregs, % of Tregs	0.148	0.600	-0.044	0.878	-0.001	0.998	-0.412	0.270
aTregs, % of Tregs	-0.371	0.173	-0.051	0.857	-0.693	0.006	-0.492	0.179
Th/CTL	0.186	0.507	-0.086	0.759	0.411	0.145	-0.200	0.607

Th1/Th2	0.256	0.357	-0.246	0.376	0.131	0.655	-0.252	0.513
Th1/Th17	0.252	0.365	-0.231	0.408	0.146	0.619	-0.305	0.426
Neutrophils/Lymphocytes	0.369	0.194	-0.083	0.779	0.318	0.290	0.425	0.254

Clinical Parameters	**ALT**		**AST**		**APTT**		**INR**	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Neutrophils, % of Leukocytes	0.139	0.651	-0.290	0.336	-0.319	0.267	0.250	0.389
Monocytes, % of Leukocytes	0.062	0.841	0.132	0.668	-0.274	0.344	0.032	0.913
Macrophages, % of Leukocytes	-0.139	0.651	-0.271	0.370	-0.471	0.089	-0.160	0.586
DC, % of Leukocytes	-0.281	0.353	-0.102	0.742	-0.362	0.203	-0.494	0.073

MDSC, % of Leukocytes	0.128	0.676	0.430	0.143	-0.041	0.889	0.843	**<0.001**
G-MDSC, % of MDSC	0.046	0.883	-0.004	0.989	0.344	0.907	-0.179	0.540
M-MDSC, % of MDSC	0.035	0.908	-0.024	0.939	0.074	0.801	0.172	0.557
G-MDSC, % of Leukocytes	0.001	0.997	0.109	0.724	-0.073	0.805	0.364	0.201
M-MDSC, % of Leukocytes	0.168	0.584	0.375	0.207	-0.008	0.979	0.713	0.004
NK cells, % of Leukocytes	-0.160	0.601	-0.143	0.228	-0.349	0.221	-0.168	0.566
NKT cells, % of Leukocytes	-0.391	0.186	-0.506	0.078	0.004	0.988	-0.090	0.760
$CD69^{+}$NK cells, % of NK cells	-0.298	0.324	-0.365	0.220	0.563	0.036	-0.129	0.662
$CD69^{+}$NKT cells, % of NKT cells	-0.094	0.761	0.155	0.614	-0.069	0.816	-0.044	0.882
B cells, % of Leukocytes	0.378	0.183	0.696	0.006	-0.104	0.713	-0.331	0.228

ns-memory B cells, % of B cells	-0.161	0.583	-0.030	0.918	0.186	0.506	-0.312	0.257
Naive B cells, % of B cells	0.248	0.392	-0.077	0.793	-0.015	0.958	-0.267	0.337
cs-memory B cells, % of B cells	-0.155	0.596	-0.138	0.639	0.018	0.949	-0.153	0.586
Plasma cells, % of B cells	0.110	0.720	-0.064	0.835	0.632	0.015	0.135	0.646
Plasma cells 1, % of B cells	-0.011	0.972	-0.073	0.813	0.758	0.002	0.054	0.855
Plasmablasts, % of B cells	0.788	**<0.001**	0.744	0.002	0.047	0.869	<0.001	>0.999
Transitional B cells, % of B cells	0.068	0.818	0.337	0.238	0.334	0.224	0.417	0.123
Bregs-1, % of Transitional B cells	-0.426	0.129	-0.330	0.249	-0.278	0.316	-0.574	0.025
Pro B cells, % of Transitional B cells	-0.228	0.434	-0.487	0.077	-0.336	0.220	-0.236	0.398
Pre B cells, % of Pro B cells	0.525	0.065	0.320	0.286	-0.250	0.389	-0.054	0.854

T cells, % of Leukocytes	-0.206	0.481	0.186	0.524	0.047	0.867	-0.104	0.711
Th, % of T cells	0.188	0.520	0.327	0.255	-0.119	0.674	0.268	0.334
Th17, % of Th	-0.150	0.610	-0.006	0.984	-0.080	0.777	0.332	0.226
Th1, % of Th	0.251	0.387	0.065	0.824	0.164	0.560	-0.328	0.232
Th2, % of Th	-0.147	0.617	-0.015	0.959	-0.146	0.605	0.270	0.331
emTh, % of Th	-0.303	0.292	-0.337	0.239	-0.169	0.547	0.368	0.178
cmTh, % of Th	-0.143	0.626	0.273	0.345	-0.051	0.857	-0.110	0.696
eTh, % of Th	0.496	0.071	0.005	0.986	0.193	0.491	0.002	0.993
nTh, % of Th	-0.158	0.590	0.212	0.468	-0.006	0.983	-0.385	0.157
aTh, % of Th	-0.181	0.535	0.010	0.973	-0.077	0.784	0.081	0.775

CTL, % of T cells	-0.195	0.505	-0.316	0.271	0.144	0.609	-0.247	0.375
emCTL, % of CTL	-0.072	0.806	0.172	0.557	-0.556	0.031	-0.028	0.921
cmCTL, % of CTL	-0.079	0.787	0.138	0.637	0.281	0.311	0.129	0.648
eCTL, % of CTL	0.105	0.722	-0.240	0.409	0.192	0.493	<0.001	0.998
nCTL, % of CTL	-0.079	0.788	0.245	0.399	0.442	0.099	-0.161	0.567
aCTL, % of CTL	-0.195	0.503	0.039	0.895	0.096	0.734	0.047	0.868
Tregs, % of Th	0.035	0.905	-0.123	0.676	0.105	0.709	0.257	0.355
mTregs, % of Tregs	-0.402	0.154	-0.338	0.238	-0.179	0.524	0.286	0.302
maTregs, % of Tregs	0.002	0.996	0.245	0.399	-0.364	0.182	-0.221	0.429
nTregs, % of Tregs	0.368	0.195	0.065	0.827	0.539	0.038	-0.059	0.835

aTregs, % of Tregs	0.222	0.445	0.163	0.577	0.1132	0.688	0.616	0.014
Th/CTL	-0.342	0.231	-0.222	0.445	0.206	0.462	0.058	0.837
Th1/Th2	0.577	0.031	0.131	0.655	0.028	0.921	-0.084	0.767
Th1/Th17	0.567	0.034	0.184	0.529	0.026	0.928	-0.129	0.646
Neutrophils/Lymphocytes	0.241	0.427	-0.345	0.248	-0.148	0.614	0.114	0.697

Clinical Parameters	**Creatinine**		**CRP**	
	r-value	p-value	r-value	p-value
Neutrophils, % of Leukocytes	-0.050	0.866	-0.004	0.990
Monocytes, % of Leukocytes	0.088	0.764	-0.163	0.631

Macrophages, % of Leukocytes	0.252	0.385	-0.147	0.666
DC, % of Leukocytes	0.376	0.185	-0.364	0.271
MDSC, % of Leukocytes	-0.068	0.817	0.672	0.024
G-MDSC, % of MDSC	-0.070	0.812	0.094	0.784
M-MDSC, % of MDSC	-0.055	0.853	-0.112	0.744
G-MDSC, % of Leukocytes	-0.119	0.685	0.734	0.010
M-MDSC, % of Leukocytes	-0.050	0.866	0.353	0.287
NK cells, % of Leukocytes	-0.020	0.946	-0.426	0.191
NKT cells, % of Leukocytes	-0.156	0.594	-0.306	0.361
$CD69^{+}$NK cells, % of NK cells	-0.258	0.374	0.182	0.592

$CD69^{+}$NKT cells, % of NKT cells	0.171	0.559	-0.042	0.902
B cells, % of Leukocytes	0.582	0.003	-0.338	0.283
ns-memory B cells, % of B cells	0.234	0.402	-0.289	0.363
Naive B cells, % of B cells	-0.471	0.084	0.102	0.752
cs-memory B cells, % of B cells	0.080	0.776	0.047	0.884
Plasma cells, % of B cells	-0.497	0.070	0.380	0.249
Plasma cells 1, % of B cells	-0.501	0.068	0.296	0.377
Plasmablasts, % of B cells	0.116	0.680	-0.145	0.653
Transitional B cells, % of B cells	-0.026	0.927	0.693	0.012
Bregs-1, % of Transitional B cells	0.308	0.264	-0.474	0.120

Pro B cells, % of Transitional B cells	-0.088	0.754	-0.209	0.515
Pre B cells, % of Pro B cells	-0.156	0.596	0.112	0.743
T cells, % of Leukocytes	0.174	0.536	0.247	0.439
Th, % of T cells	0.123	0.661	0.079	0.808
Th17, % of Th	0.415	0.124	0.029	0.929
Th1, % of Th	-0.437	0.104	-0.134	0.679
Th2, % of Th	0.419	0.121	0.094	0.772
emTh, % of Th	0.047	0.867	0.497	0.100
cmTh, % of Th	0.300	0.279	-0.546	0.066
eTh, % of Th	-0.580	0.024	0.242	0.448

nTh, % of Th	0.488	0.065	-0.368	0.240
aTh, % of Th	0.441	0.100	-0.024	0.941
CTL, % of T cells	-0.089	0.751	-0.073	0.821
emCTL, % of CTL	0.332	0.227	-0.275	0.386
cmCTL, % of CTL	-0.102	0.719	0.174	0.589
eCTL, % of CTL	-0.214	0.445	0.176	0.585
nCTL, % of CTL	0.059	0.834	-0.069	0.831
aCTL, % of CTL	0.183	0.515	0.186	0.563
Tregs, % of Th	-0.093	0.742	0.101	0.755
mTregs, % of Tregs	-0.126	0.654	0.274	0.388

maTregs, % of Tregs	0.706	0.003	-0.440	0.152
nTregs, % of Tregs	-0.594	0.020	0.263	0.408
aTregs, % of Tregs	-0.016	0.956	0.539	0.071
Th/CTL	-0.160	0.570	-0.246	0.441
Th1/Th2	-0.514	0.050	-0.049	0.880
Th1/Th17	-0.494	0.061	-0.065	0.841
Neutrophils/Lymphocytes	-0.247	0.395	-0.315	0.345

Supplementary Table 6. Multiple comparison statistical calculations between immune cells and UICC staging. Abbreviations: aCTL: Activated cytotoxic T lymphocytes; aTh: Activated helper T cells; aTregs: Activated Tregs; Bregs: Regulatory B cells; cmCTL: Central memory cytotoxic T lymphocytes; cmTh: Central memory helper T cells; CTL: Cytotoxic T lymphocytes; cs-memory B cells: Class-switched memory B cells; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; eTh: Effector helper T cells; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; G-MDSC: Granulocyte-like MDSC; pro B: Progenitor B cells; pre B: Precursor B cells; MDSC: Myeloid-derived suppressor cells; M-MDSC: Monocyte-like MDSC; mTregs: Memory Tregs; maTregs: Memory-activated Tregs; ns-memory B cells: non-class-switched memory B cells; nCTL: Naïve cytotoxic T lymphocytes; nTh: Naïve helper T cells; nTregs: Naive Tregs; NK: Natural killer cells; NKT: Natural killer T cells; Tregs: Regulatory T cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells. PreOP: Pre-operation; POM 3: Postoperative month 3; POM 12: Postoperative month 12; SD: Standard deviation; N: Number of patients; ***: $p < 0.001$.

Cell Type	Staging 1 VS. Staging 2	Staging 1 VS. Staging >2	Staging 2 VS. Staging >2
	p-value	p-value	p-value
Neutrophils, % of Leukocytes	0.827	>0.999	0.774

Monocytes, % of Leukocytes	>0.999	>0.999	>0.999
Macrophages, % of Leukocytes	>0.999	0.859	>0.999
DC, % of Leukocytes	0.165	>0.999	0.123
MDSC, % of Leukocytes	0.338	>0.999	0.325
G-MDSC, % of MDSC	>0.999	0.886	>0.999
M-MDSC, % of MDSC	>0.999	0.344	0.415
G-MDSC, % of Leukocytes	0.627	0.899	>0.999
M-MDSC, % of Leukocytes	0.691	0.670	0.164

NK cells, % of Leukocytes	0.563	0.164	>0.999
NKT cells, % of Leukocytes	>0.999	0.170	0.332
$CD69^{+}$NK cells, % of NK cells	>0.999	>0.999	>0.999
$CD69^{+}$NKT cells, % of NKT cells	0.616	>0.999	0.400
B cells, % of Leukocytes	0.014	>0.999	0.021
ns-memory B cells, % of B cells	>0.999	>0.999	0.918
Naive B cells, % of B cells	>0.999	>0.999	>0.999
cs-memory B cells, % of B cells	>0.999	>0.999	>0.999

Plasma cells, % of B cells	>0.999	>0.999	>0.999
Plasma cells 1, % of B cells	>0.999	>0.999	>0.999
Plasmablasts, % of B cells	0.829	>0.999	>0.999
Transitional B cells, % of B cells	>0.999	0.893	>0.999
Bregs-1, % of Transitional B cells	0.201	>0.999	0.499
Pro B cells, % of Transitional B cells	>0.999	0.624	0.809
Pre B cells, % of Pro B cells	>0.999	>0.999	>0.999
T cells, % of Leukocytes	0.705	>0.999	0.392

Th, % of T cells	>0.999	>0.999	>0.999
Th17, % of Th	0.691	0.042	0.417
Th1, % of Th	>0.999	0.076	0.312
Th2, % of Th	0.764	0.182	>0.999
emTh, % of Th	0.056	0.220	>0.999
cmTh, % of Th	0.246	>0.999	0.450
eTh, % of Th	>0.999	0.329	0.285
nTh, % of Th	0.114	>0.999	0.193

aTh, % of Th	>0.999	0.702	>0.999
CTL, % of T cells	>0.999	>0.999	>0.999
emCTL, % of CTL	>0.999	>0.999	0.970
cmCTL, % of CTL	>0.999	0.762	>0.999
eCTL, % of CTL	>0.999	>0.999	0.818
nCTL, % of CTL	>0.999	>0.999	>0.999
aCTL, % of CTL	>0.999	0.857	>0.999
Tregs, % of Th	0.773	>0.999	0.588

mTregs, % of Tregs	0.241	>0.999	0.191
maTregs, % of Tregs	0.036	0.125	0.003
nTregs, % of Tregs	>0.999	>0.999	0.925
aTregs, % of Tregs	0.589	>0.999	>0.999
Th/CTL	>0.999	0.546	0.682
Th1/Th2	>0.999	0.102	0.242
Th1/Th17	>0.999	0.040	0.141
Neutrophils/Lymphocytes	0.500	0.994	0.179

Supplementary Table 7. Statistical calculation on all detected subsets at different time points. Abbreviations: aCTL: Activated cytotoxic T lymphocytes; aTh: Activated helper T cells; aTregs: Activated Tregs; Bregs: Regulatory B cells; cmCTL: Central memory cytotoxic T lymphocytes; cmTh: Central memory helper T cells; CTL: Cytotoxic T lymphocytes; cs-memory B cells: Class-switched memory B cells; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; eTh: Effector helper T cells; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; G-MDSC: Granulocyte-like MDSC; pro B: Progenitor B cells; pre B: Precursor B cells; MDSC: Myeloid-derived suppressor cells; M-MDSC: Monocyte-like MDSC; mTregs: Memory Tregs; maTregs: Memory-activated Tregs; ns-memory B cells: non-class-switched memory B cells; nCTL: Naïve cytotoxic T lymphocytes; nTh: Naïve helper T cells; nTregs: Naive Tregs; NK: Natural killer cells; NKT: Natural killer T cells; Tregs: Regulatory T cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells. PreOP: Pre-operation; POM 3: Postoperative month 3; POM 12: Postoperative month 12; SD: Standard deviation; N: Number of patients; ***: $p < 0.001$.

Cell Type	PreOP (Mean±SD, N=15)	POM 3 (Mean±SD, N=15)	POM 12 (Mean±SD, N=15)	Total p-value
Neutrophils, % of Leukocytes	59.11±16.15	63.50±14.00	54.48±12.08	0.633

Monocytes, % of Leukocytes	5.51±2.34	5.59±2.74	6.92±3.75	0.486
Macrophages, % of Leukocytes	0.44±0.43	0.88±0.83	0.49±0.71	0.358
DC, % of Leukocytes	0.18±0.14	0.21±0.23	0.36±0.47	0.127
MDSC, % of Leukocytes	1.26±0.91	1.68±1.39	1.71±1.53	0.526
G-MDSC, % of MDSC	18.91±18.64	24.14±21.08	49.09±29.93	**<0.001**
M-MDSC, % of MDSC	57.16±25.84	46.56±34.35	22.36±26.01	**<0.001**
G-MDSC, % of Leukocytes	0.18±0.17	0.24±0.26	0.92±1.11	0.021
M-MDSC, % of Leukocytes	0.78±0.68	1.00±1.21	0.27±0.36	0.014

NK cells, % of Leukocytes	2.57±2.12	3.13±2.93	2.55±1.87	0.505
NKT cells, % of Leukocytes	0.10±0.11	0.15±0.19	0.20±0.32	0.314
$CD69^{+}$NK cells, % of NK cells	1.46±1.32	2.85±3.32	2.48±2.19	0.212
$CD69^{+}$NKT cells, % of NKT cells	11.83±8.51	12.12±13.92	11.66±16.71	0.923
B cells, % of Leukocytes	2.51±1.32	3.82±5.54	4.39±4.99	0.215
ns-memory B cells, % of B cells	8.81±16.63	4.29±8.74	4.46±6.55	0.129
Naive B cells, % of B cells	36.91±22.03	33.93±27.33	40.00±23.97	0.761
cs-memory B cells, % of B cells	6.51±5.52	3.82±4.78	1.55±2.19	0.028

Plasma cells, % of B cells	1.49±2.43	1.65±2.39	0.93±1.86	0.302
Plasma cells 1, % of B cells	3.07±4.95	2.26±1.97	2.68±2.25	0.573
Plasmablasts, % of B cells	0.26±0.53	0.14±0.16	0.13±0.26	0.386
Transitional B cells, % of B cells	10.50±12.39	10.59±5.63	13.17±9.85	0.688
Bregs-1, % of Transitional B cells	7.99±12.21	5.65±6.61	5.25±6.99	0.664
Pro B cells, % of Transitional B cells	30.80±25.70	31.68±23.49	23.62±23.52	0.682
Pre B cells, % of Pro B cells	56.70±40.47	63.74±40.47	43.67±36.04	0.346
T cells, % of Leukocytes	21.26±8.18	18.65±9.40	21.33±9.35	0.484

Th, % of T cells	67.55±15.76	66.87±16.16	65.83±18.85	0.663
Th17, % of Th	17.82±6.58	17.35±7.23	15.91±7.55	0.526
Th1, % of Th	48.09±14.84	49.92±12.79	51.07±15.90	0.509
Th2, % of Th	12.23±4.49	14.40±5.46	12.85±5.79	0.310
emTh, % of Th	54.96±15.00	51.66±16.66	50.48±18.07	0.558
cmTh, % of Th	15.66±9.53	17.51±11.86	20.14±11.18	0.564
eTh, % of Th	17.45±16.02	15.76±13.09	11.22±8.07	0.357
nTh, % of Th	11.92±11.72	15.06±9.64	18.12±14.31	0.204

aTh, % of Th	1.58±0.68	3.09±3.93	2.70±2.50	0.263
CTL, % of T cells	27.42±15.28	27.77±14.79	27.88±15.33	0.878
emCTL, % of CTL	42.49±16.54	39.57±12.35	45.46±11.38	0.334
cmCTL, % of CTL	8.06±9.77	8.09±10.12	6.12±4.43	0.574
eCTL, % of CTL	43.96±22.39	44.88±17.52	40.42±9.56	0.615
nCTL, % of CTL	5.49±4.87	7.47±8.01	8.00±5.65	0.300
aCTL, % of CTL	3.63±3.46	4.88±4.51	7.47±11.06	0.294
Tregs, % of Th	8.97±4.69	13.39±11.20	11.82±5.41	0.246

mTregs, % of Tregs	65.00±10.51	63.47±13.53	63.29±11.06	0.678
maTregs, % of Tregs	19.89±11.09	18.62±8.90	20.42±10.08	0.770
nTregs, % of Tregs	14.88±10.94	16.49±12.15	15.37±11.41	0.691
aTregs, % of Tregs	0.23±0.14	1.42±4.40	0.92±1.90	0.324
Th/CTL	5.12±6.52	3.79±2.87	3.50±2.50	0.336
Th1/Th2	5.50±6.30	4.15±2.41	5.20±3.50	0.487
Th1/Th17	3.86±4.06	3.90±3.39	4.62±3.78	0.623
Neutrophils/Lymphocytes	3.49±1.73	2.50±4.08	2.50±1.45	0.429

Supplementary Table 8. Multiple comparison statistical calculations on all detected subsets at different time points. Abbreviations: aCTL: Activated cytotoxic T lymphocytes; aTh: Activated helper T cells; aTregs: Activated Tregs; Bregs: Regulatory B cells; cmCTL: Central memory cytotoxic T lymphocytes; cmTh: Central memory helper T cells; CTL: Cytotoxic T lymphocytes; cs-memory B cells: Class-switched memory B cells; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; eTh: Effector helper T cells; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; G-MDSC: Granulocyte-like MDSC; pro B: Progenitor B cells; pre B: Precursor B cells; MDSC: Myeloid-derived suppressor cells; M-MDSC: Monocyte-like MDSC; mTregs: Memory Tregs; maTregs: Memory-activated Tregs; ns-memory B cells: non-class-switched memory B cells; nCTL: Naïve cytotoxic T lymphocytes; nTh: Naïve helper T cells; nTregs: Naive Tregs; NK: Natural killer cells; NKT: Natural killer T cells; Tregs: Regulatory T cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells. PreOP: Pre-operation; POM 3: Postoperative month 3; POM 12: Postoperative month 12; SD: Standard deviation; N: Number of patients; ***: $p < 0.001$.

Cell Type	PreOP VS. POM 3	PreOP VS. POM 12	POM 3 VS. POM 12
	p-value	p-value	p-value
Neutrophils, % of Leukocytes	>0.999	>0.999	>0.999

Monocytes, % of Leukocytes	>0.999	>0.999	0.775
Macrophages, % of Leukocytes	0.495	>0.999	>0.999
DC, % of Leukocytes	>0.999	0.482	0.131
MDSC, % of Leukocytes	0.562	>0.999	>0.999
G-MDSC, % of MDSC	0.926	0.003	0.003
M-MDSC, % of MDSC	0.851	**<0.001**	0.003
G-MDSC, % of Leukocytes	>0.999	0.043	0.100
M-MDSC, % of Leukocytes	>0.999	0.017	0.029

NK cells, % of Leukocytes	0.839	>0.999	>0.999
NKT cells, % of Leukocytes	0.945	0.839	>0.999
CD69+NK cells, % of NK cells	0.264	0.509	>0.999
CD69+NKT cells, % of NKT cells	>0.999	>0.999	>0.999
B cells, % of Leukocytes	>0.999	0.384	0.708
ns-memory B cells, % of B cells	0.266	0.471	>0.999
Naive B cells, % of B cells	>0.999	>0.999	>0.999
cs-memory B cells, % of B cells	0.612	0.010	0.196

Plasma cells, % of B cells	>0.999	0.574	0.191
Plasma cells 1, % of B cells	>0.999	>0.999	>0.999
Plasmablasts, % of B cells	>0.999	0.437	>0.999
Transitional B cells, % of B cells	>0.999	>0.999	>0.999
Bregs-1, % of Transitional B cells	>0.999	>0.999	>0.999
Pro B cells, % of Transitional B cells	>0.999	>0.999	>0.999
Pre B cells, % of Pro B cells	>0.999	>0.999	0.651
T cells, % of Leukocytes	0.256	>0.999	>0.999

Th, % of T cells	>0.999	>0.999	>0.999
Th17, % of Th	>0.999	0.481	>0.999
Th1, % of Th	>0.999	0.280	>0.999
Th2, % of Th	0.522	>0.999	0.994
emTh, % of Th	>0.999	>0.999	>0.999
cmTh, % of Th	>0.999	0.834	>0.999
eTh, % of Th	>0.999	0.435	0.743
nTh, % of Th	>0.999	0.312	>0.999

aTh, % of Th	0.456	0.414	>0.999
CTL, % of T cells	>0.999	>0.999	>0.999
emCTL, % of CTL	>0.999	>0.999	0.123
cmCTL, % of CTL	>0.999	>0.999	>0.999
eCTL, % of CTL	>0.999	>0.999	0.590
nCTL, % of CTL	>0.999	0.193	>0.999
aCTL, % of CTL	0.822	0.715	>0.999
Tregs, % of Th	0.508	0.325	>0.999

mTregs, % of Tregs	>0.999	>0.999	>0.999
maTregs, % of Tregs	>0.999	>0.999	>0.999
nTregs, % of Tregs	>0.999	>0.999	>0.999
aTregs, % of Tregs	0.945	0.553	>0.999
Th/CTL	>0.999	0.967	>0.999
Th1/Th2	>0.999	>0.999	0.704
Th1/Th17	>0.999	0.521	>0.999
Neutrophils/Lymphocytes	>0.999	>0.999	>0.999

Supplementary Table 9. Statistical calculations at different time points for all detected subgroups between recurrence and no-recurrence patients. Abbreviations: aCTL: Activated cytotoxic T lymphocytes; aTh: Activated helper T cells; aTregs: Activated Tregs; Bregs: Regulatory B cells; cmCTL: Central memory cytotoxic T lymphocytes; cmTh: Central memory helper T cells; CTL: Cytotoxic T lymphocytes; cs-memory B cells: Class-switched memory B cells; DC: Dendritic cells; eCTL: Effector cytotoxic T lymphocytes; eTh: Effector helper T cells; emCTL: Effector memory cytotoxic T lymphocytes; emTh: Effector memory helper T cells; G-MDSC: Granulocyte-like MDSC; pro B: Progenitor B cells; pre B: Precursor B cells; MDSC: Myeloid-derived suppressor cells; M-MDSC: Monocyte-like MDSC; mTregs: Memory Tregs; maTregs: Memory-activated Tregs; ns-memory B cells: non class-switched memory B cells; nCTL: Naïve cytotoxic T lymphocytes; nTh: Naïve helper T cells; nTregs: Naive Tregs; NK: Natural killer cells; NKT: Natural killer T cells; Tregs: Regulatory T cells; Th: Helper T cells; Th1: Type 1 helper T cells; Th2: Type 2 helper T cells; Th17: Type 17 helper T cells. PreOP: Pre-operation; POM 3: Postoperative month 3; POM 12: Postoperative month 12; N: Number of patients; VS: Versus. R: Recurrence after operation; NR: No recurrence after operation. ***: $p < 0.001$.

Cell Type	PreOP	POM 3	POM 12
	N (R: 9 VS. NR: 6), p-value	N (R: 9 VS. NR: 6), p-value	N (R: 9 VS. NR: 6), p-value
Neutrophils, % of Leukocytes	0.852	0.776	>0.999

Monocytes, % of Leukocytes	0.662	0.776	0.864
Macrophages, % of Leukocytes	0.638	0.285	0.047
DC, % of Leukocytes	0.295	0.628	0.111
MDSC, % of Leukocytes	0.662	0.776	0.456
G-MDSC, % of MDSC	0.020	0.955	0.456
M-MDSC, % of MDSC	0.181	0.456	0.607
G-MDSC, % of Leukocytes	0.022	0.436	0.224
M-MDSC, % of Leukocytes	0.414	0.864	0.665

NK cells, % of Leukocytes	>0.999	0.181	0.066
NKT cells, % of Leukocytes	0.298	0.031	0.312
$CD69^{+}$NK cells, % of NK cells	0.142	0.979	0.088
$CD69^{+}$NKT cells, % of NKT cells	0.151	0.149	0.214
B cells, % of Leukocytes	0.888	0.456	0.955
ns-memory B cells, % of B cells	0.456	0.955	0.864
Naive B cells, % of B cells	0.026	0.475	0.328
cs-memory B cells, % of B cells	0.328	>0.999	0.529

Plasma cells, % of B cells	0.823	0.836	0.456
Plasma cells 1, % of B cells	0.950	0.664	0.145
Plasmablasts, % of B cells	0.885	0.709	0.667
Transitional B cells, % of B cells	0.776	0.328	0.955
Bregs-1, % of Transitional B cells	0.325	0.221	0.975
Pro B cells, % of Transitional B cells	0.864	0.145	0.224
Pre B cells, % of Pro B cells	0.181	0.526	0.926
T cells, % of Leukocytes	0.224	0.388	0.272

Th, % of T cells	0.285	0.272	0.272
Th17, % of Th	0.224	0.529	0.114
Th1, % of Th	0.456	>0.999	0.456
Th2, % of Th	0.607	0.026	0.456
emTh, % of Th	0.529	0.864	0.456
cmTh, % of Th	0.313	0.181	0.456
eTh, % of Th	0.607	0.066	0.529
nTh, % of Th	0.456	>0.999	0.272

aTh, % of Th	0.529	>0.999	>0.999
CTL, % of T cells	0.181	0.181	0.272
emCTL, % of CTL	0.955	0.049	0.066
cmCTL, % of CTL	0.955	0.049	0.181
eCTL, % of CTL	0.955	0.036	0.272
nCTL, % of CTL	>0.999	0.689	0.272
aCTL, % of CTL	>0.999	0.607	0.978
Tregs, % of Th	0.181	0.066	0.272

mTregs, % of Tregs	0.529	0.456	>0.999
maTregs, % of Tregs	0.607	0.066	>0.999
nTregs, % of Tregs	0.607	0.145	0.456
aTregs, % of Tregs	0.171	0.023	0.475
Th/CTL	0.114	0.145	0.272
Th1/Th2	0.456	0.083	0.456
Th1/Th17	0.372	0.607	0.272
Neutrophils/Lymphocytes	0.282	0.955	0.776

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