Aus dem

CharitéCentrum 8 für Chirurgische Medizin

Chirurgische Klinik

Campus Charité Mitte | Campus Virchow-Klinikum

Direktor: Professor Dr. med. Johann Pratschke

**Habilitationsschrift**

# The clinical significance of the host immunologic competence and related angiogenesis and necrosis in the tumor microenvironment of hepatobiliary cancer

zur Erlangung der Lehrbefähigung
für das Fach Chirurgie

vorgelegt dem Fakultätsrat der Medizinischen Fakultät
Charité-Universitätsmedizin Berlin

von

**Dr. med. Georgi Atanasov**

**Eingereicht:** **Januar 2020**

**Dekan:** **Prof. Dr. Axel R. Pries**

**1. Gutachter/in:** **Prof. Dr. Roland S. Croner**

**2. Gutachter/in:** **Prof. Dr. Thomas Becker**

**Table of contents**

**ABBREVIATIONS**

| | |
|---|---|
| AFP | Alpha-Fetoprotein |
| ANG | Angiopoietin |
| CCA | Cholangiocarcinoma |
| EGFR | epidermal growth factor receptor |
| FDA | U. S. Food and Drug Administration |
| HCC | Hepatocellular carcinoma |
| HBV | Hepatitis B |
| HCV | Hepatitis C |
| ICC | Intrahepatic cholangiocarcinoma |
| MDSC | Myeloid-derived suppressor cells |
| NASH | Nonalcoholic steatohepatitis |
| PDAC | Periductal adenocarcinoma of the pancreas |
| PD-1 | Programmed cell death protein 1 |
| PHC | Perihilar cholangiocarcinoma |
| TACE | Transarterial chemoembolization |
| TAMs | Tumor-associated macrophages |
| TCA | Tumor central area |
| TIF | Tumor infiltrating front |
| TEMs | TIE2-expressing monocytes |
| TNT | Tumor necrosis therapy |
| Tregs | Regulatory T cells |
| VEGF | Vascular endothelial growth factor |

## 1. INTRODUCTION

The term hepatobiliary cancer describes primary tumors originating in the liver or the intra- or extrahepatic biliary ductal system. Primary cancer of the hepatobiliary tract comprises highly aggressive tumor entities with median patient survival rates of less than 12 months in untreated cases (1-3). Hepatobiliary cancer consists of hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and perihilar cholangiocarcinoma (PHC). Formally, adenocarcinomas of the gall bladder, distal bile duct and periampullary region belong to biliary cancer, as well. However, these entities shall be of no further discussion in the current work, because focus was set on the more common HCC, ICC and PHC.

Nowadays, surgical tumor resection with pathohistological negative resection margins (R0 resection), orthotopic liver transplantation (LTX) or radiofrequency ablation in selected cases represent the only available realistic chance of a curative treatment (4). Unfortunately, only a limited fraction of the patients qualifies for surgical therapies at the time of diagnosis. For advanced or metastatic disease the state of the art standard of care consists of tyrosine kinase inhibitors (such as sorafenib for HCC) or gemcitabine-based chemotherapy (for cancer arising from the epithelium of the bile duct) (5, 6). In addition, promising results have been observed with immunotherapy utilizing nivolumab-based checkpoint inhibition of the cellular programmed cell death protein 1 (PD-1). This regime was recently ratified in the USA (7). In addition to that, no established standard second-line therapy option exists. Therefore, there is unmet need for novel diagnostic and therapeutic modalities aiming to improve the management of hepatobiliary cancer.

Immunogenicity constitutes an integrational part of the biology of hepatobiliary cancer. The causal immunologic involvement of the host in the etiology of these tumor entities renders the immune system an attractive target for novel therapeutic modalities and promising diagnostic tools. Immune-based approaches in solid human cancer have shown promising results in recent years, especially in combination with established chemotherapy protocols (8-10). A genuine next-level frontier for immune checkpoint inhibition is to demonstrate

efficacy in diseases that have so far proved refractory, including hepatobiliary cancer. Thus, there is an increasing demand for basic research in regard of the host immunologic competence and related features of the tumor microenvironment, which will help deliver novel molecular and cellular targets of immune modulation.

### 1.1. Prognosis and management of primary hepatobiliary cancer

#### 1.1.1. Hepatocellular carcinoma

HCC is the most common primary liver malignancy. It arises on grounds of a chronic liver inflammatory disease and represents one of the most frequent cancer-related mortalities (11). Most of HCC occur in an established hepatic environment of chronic inflammation, commonly due to alcoholic liver cirrhosis or viral hepatitis B (HBV) and C (HCV) (12-14). Recent data demonstrated also that nonalcoholic steatohepatitis (NASH) is an important factor causally involved in hepatocarcinogenesis (15). A large number of the HCC patients present at the time of diagnosis with an intermediate or advanced stage HCC, which deprives them of the chance of curative therapy. Recent studies indicated the mean survival time for patients with an advanced HCC to be as low as 10 months (16).

Liver resection constitutes the state of the art curative treatment strategy for solitary HCC in compensated CHILD A liver cirrhosis (17). When conforming to these criteria, the liver failure incidence, patient death and outcome can be improved to a 5-year overall survival of 70% in high volume centers (18). However, in patients with an underlying hepatic dysfunction the advantage of a major hepatectomy should be weight against impending complications in the postoperative course. LTX delivers the chance of eliminating the primary hepatic malignancy and, at the same time, cure the underlying liver cirrhosis that represents a major peril for HCC. However, an important disadvantage in this setting is the ubiquitous donor organ shortage resulting in high waiting list mortality rates (19). Applying the Milan criteria (a single lesion ≤5 cm, or no more than three nodules, each ≤3 cm, absence of vascular involvement

or metastatic spread) for graft allocation can lead to a marked increase in overall survival: 1-, 3-, 5-, and 10-year survival of 89.7, 83.7, 75.8, and 62.1%, respectively (20).

In HCC patients exceeding the Milan criteria, transarterial chemoembolization (TACE) belongs to the most common tools that are applied to achieve a disease control and even downstaging in order to retrieve criteria eligible for LTX. TACE can prolong the median survival of HCC patients from 16 to 20 months, however, the extent of improvement depends strongly on the individual patient characteristics, such as tumor stage and biology, liver function and general health status (21). Systemic molecular targeted therapy consisting of sorafenib, an oral multikinase inhibitor, with or without concomitant TACE, represents the systemic state of the art therapeutic modality in cases not eligible for LTX or major resection but with an advanced HCC and compensated liver function (22). Treatment with sorafenib can improve median survival up to 11 months (23). However, most of the HCC patients are diagnosed with terminal stage HCC, which excludes major surgery or TACE/sorafenib treatment modalities. In these cases, when best supportive care is applied, median survival rates can reach 9 months (24).

### 1.1.2. Bile duct cancer

Cholangiocarcinoma (CCA) represents the second most frequent cancer originating from the epithelium of the bile ducts in the liver or extrahepatic, and its occurrence in western countries has risen markedly over the past decades. CCA translates into a fulminant prognosis. Importantly, concerning the specific anatomical location of the lesion (intrahepatic, perihilar or distal), distinct diagnostic and therapeutic management is conducted. Surgery, either in the form of a major hepatic resection or LTX in selected cases, is the only curative treatment option. However, most of the CCA patients present at a late and advanced tumor stage that renders curative treatment approaches impossible. Median survival is reported to be no longer than 286 days for patients with advanced and multifocal CCA (25).

#### 1.1.2.1. Intrahepatic cholangiocarcinoma

Per definition, ICC is localized proximally to the second degree ramifications of the bile duct (26). Hepatectomy with tumor free resection margins (R0) is essential for an improved prognosis. However, this can be accomplished in only 30% of the cases (27). Patients' survival within the intention-to-treat concept comprising tumors that appear resectable on preoperative imaging is 36 months (28). Multifocal ICC, liver cirrhosis, lymph node involvement and positive tumor margins (R1/R2) correlate with poor prognosis in CCA patients (27-29).

LTX is not considered a reliable option for ICC (26). Noteworthy, the 1- and 5-year cumulative risk of recurrent disease after LTX in patients with mixed hepatocellular-cholangiocellular malignancies is 42% and 65%, respectively (30). Locoregional therapy is considered to have the potential to be a reasonable palliative concept; however, a validation in high-quality studies is still to come. Nevertheless, current data suggests a potential survival benefit for ICC patients who are treated with TACE (12-15 months overall survival, compared to 3-3.5 months in cases under best supportive care) (31-33). Moreover, recent data demonstrates that TACE with drug-eluting beads has a comparable efficacy, when compared with systemic chemotherapy (11.7 vs. 11 months overall survival, respectively), and might even outperform conventional TACE (5.7 months overall survival) (34). Overall survival with state of the art systemic targeted chemotherapy can reach 8-12 months (35).

#### 1.1.2.1. Perihilar cholangiocarcinoma (Klatskin tumor)

PHC is defined as bile duct cancer that is localized distally the second degree ramifications and proximally the confluence of ductus cysticus and ductus hepaticus communis (26). Novel staging criteria have been recently introduced to help identify patients that might benefit from surgical therapy (36). Treatment with curative intent often encompasses patient

preconditioning with portal vein embolization for hypertrophy induction of the future liver remnant. Biliary tract stenting prior to surgery is also very common. Curative surgery for PHC is complex. The approach often consists of extended lobectomy, bile duct resection, vascular reconstruction after portal vein resection, Roux-en-Y hepaticojejunostomy and regional lymphadenectomy, and can be accompanied by a substantial patient morbidity in the postoperative course. Novel strategies incorporating ligation of the right branch of the portal vein in combination with in situ split of the parenchyma may lead to a rapid hypertrophy of the future left liver remnant volume and facilitate two-staged extended right lobectomy, however, this setting requires a further evaluation due to the substantial morbidity and mortality (37). In experienced high volume centers, overall survival may reach 27-47% 5-years after resection when R0 situation is accomplished (37, 38, 39).

Due to the overwhelming donor organ shortage, LTX is not considered a primary therapeutic option for PHC. In addition, the criteria eligible for LTX (unresectable PHC, < 3 cm in size, absence of lymph node dissemination or metastatic spread to distant sites) are met only by a small number of the cases, which then may translate into survival rates comparable with other common indications for LTX (40, 41). According to the current guidelines of the German Medical Board, LTX for PHC should be performed only in clinical trials. Currently, the Charité Department of Surgery supervises the ongoing pro-duct002 study (DRKS00013276), which investigates the microscopic tumor free resection margins after LTX for PHC. However, in most cases PHC patients are not eligible for curative surgery or LTX. In this scenario, the patients can be evaluated for a chemotherapy consisting of gemcitabine alone or in combination with cisplatin. Recent data demonstrated higher objective rate of responsiveness to chemotherapy (31%) and prolonged progression-free period (5.9 months) when erlotinib, a blocker of the epidermal growth factor receptor (EGFR), accompanies the chemotherapy protocol (42).

Personalized management of an advanced hepatobiliary cancer offers the opportunity to substantially improve the treatment of cancer. Distinct cancer-related molecular pathways

may qualify as promising therapy targets (43). Moreover, combination therapies comprising established chemotherapeutics and checkpoint inhibitors against growth factors or angiogenesis pathways demonstrated promising results (44). Thus, a deeper understanding of the tumor biology involved in hepatocarcinogenesis and the identification of reliable biomarkers and immunologic checkpoint inhibition targets is essential and may facilitate a personally-tailored medicine for future management of hepatobiliary cancer.

### 1.2. Tumor immunology

The role of the host immune system concerning the tumor progression has been generally underestimated in the last decades. The major breakthroughs in oncology were related to an improved understanding of genetic and molecular functions, essential for the progression of cancer that could be deployed as effective targeted therapies. However, the results from numerous clinical trials revealed that these therapies are effective only in a limited number of the tumor patients, demonstrating that significant facets of the puzzle are still missing. The clinical achievements utilizing efficient immunotherapy are built upon several major insights: (1) in the tumor microenvironment, non-tumor factors and infiltrates of host origin heavily affect tumor progression, patient survival and prognosis; (2) human cancer is characterized by an exceptional immunologic heterogeneity; (3) immunologic tumor escape mechanisms are deployed to promote cancer growth; (4) simultaneous targeting of multiple pathways facilitates successful anticancer treatment.

#### 1.2.1. Cancer-related inflammation in the tumor microenvironment

The tumor microenvironment constitutes not only of malignant cells but comprises also a complex desmoplastic multicellular tissue reaction that influences the tumor progression in a profound way (45). Among these cellular infiltrates related to the tumor microenvironment, the immune components of the host innate and adaptive immune system play the most

important roles. Tumor-associated macrophages (TAMs), T and B lymphocytes, natural killer cells, and neutrophils are integrative structural components of the tumor microenvironment. TAMs can built up to 50% of the immunologic cellular mass in solid tumors and exert a tremendous impact on tumor progression (46). Of important note, TAMs is only a preamble for various monocytes/macrophages subpopulations with distinct polarizations states and functionalities.

In simplified terms, TAMs can be ascribed to a classically activated and proinflammatory mode (M1) or to an alternatively activated, anti-inflammatory and immunosuppressive M2 state (47). TAMs exert central and ubiquitous functionalities in the scope of chronic hepatic inflammation, injury and carcinogenesis, which include activation of hepatic stellate cells mediating fibrogenesis, secretion of various metalloproteases, cytokines, growth factors and chemokines, potentiating tumor angiogenesis and progression (48, 49). Moreover, TAMs are in a constant interaction with the cancer cells, respond to tumor milieu-derived factors and act as a potent suppressor of the host adaptive immunity. Recent data revealed that TAMs

express elevated PD-L1 densities that potently inhibit the anti-tumor immune competence of T cells (50).

The immunosuppressive functions of TAMs are strengthened by a constant entanglement with cellular suppressors of myeloid origin and T regulatory lymphocytes (Tregs). The presence of the latter in solid cancer heavily affects tumor progression, as well (51). A unique subpopulation of these functionally distinct myeloid progenies that express the tyrosine kinase receptor TIE2 (TEMs) act as potent paracrine inducers of tumor angiogenesis and progression (52). In experimental cancer models, TEMs were characterized by a strong M2 polarization state, profound angiogenic activity and the genetic ablation of the TIE2 receptor thoroughly prevented the tumor angiogenesis (52). TIE2 is specifically activated by potent

factors of angiogenesis, like angiopoietin-1 and -2, and is a key modulator of adult angiogenesis and lymphangiogenesis (53). TEMs showed *in vitro* a directed angiopoietin-2-driven migration, revealing a homing mechanism for TEMs to cancerous tissues (54). Furthermore, TEMs are preferentially detected in human tumors but not in healthy tissues, form tight clusters with Tregs and facilitate a generation of suppressive $CD25^{+}FoxP3^{+}CD4^{+}$ lymphocytes from the $CD4^{+}$ T phenotype (54, 55).

### 1.2.2. Tumor angiogenesis

To sustain accelerated proliferation, malignant cells have to urgently mediate the sprouting of a new vascular network, which is enabled by elevated levels of established factors of angiogenesis, like angiopoietins and vascular endothelial growth factor (VEGF), which are secreted by malignant and stromal cells in the tumor microenvironment (56). Angiopoietin-1 and -2 levels are significantly increased in plasma of tumor patients, when compared with healthy individuals (57, 58). Furthermore, angiopoietin-1 and -2 have been demonstrated to be overexpressed in cancerous tissues and to promote aberrant angiogenesis (59, 60). In the course of this ‘’angiogenic switch’’, the enhanced activation of the angiopoietin-TIE2-axis leads to an increased tumor angiogenesis and invasion with proangiogenic myeloid precursors, especially at the tumor infiltrating front (TIF) (61).

Distorted homeostasis in the angiopoietin-1 and -2 signaling pathways is crucial for tumor progression, due to the partly opposite functionalities. Angiopoietin-1 is essential in mediating vessel maturation, adhesion, migration and survival of endothelial cells, however, cumulating novel data delineated its boosting effect on cancer growth, as well (62). Angiopoietin-2, on the other side, disrupts the integrity between endothelial and perivascular cells to specifically promote tumor angiogenesis and metastasis (60).

### 1.2.3. Occurrence of histologic tumor necrosis

Immunologic infiltration with immune competent cells has been mechanistically linked to the formation of tissue necrosis in the tumor microenvironment (63). In addition, the occurrence of histologic tumor necrosis has been identified as a novel biomarker indicating poor patient outcome (64). Various TAMs subpopulations and related molecular pathways have been identified as the key mediators in the process of necrosis formation (65). Furthermore, emerging experimental and clinical data highlighted tumor necrosis as the hallmark of cancer progression and delineated its association with tumor angiogenesis and infiltrating TAMs (66).

Data on the importance of tumor necrosis in primary hepatobiliary tumors is scarce. Several mechanisms have been proposed to shed light on the formation of necrosis in the tumor microenvironment. In the classic scenario, the rapid progression of cancer outgrows its own blood supply and inevitably leads to compromised tissue oxygen delivery, which results in necrotic cell death. However, novel mechanisms demonstrated deeper complexity and linked necrosis formation to chronic inflammation, silencing of tumor-suppressor genes, increased

microthrombosis and mutation of proto-oncogenes, which ultimately fosters tumor progression (67).

### 1.2.4. Clinical significance

Novel therapeutic options for patients with hepatobiliary malignancies represent an urgent unmet need. On grounds of their fundamental role in tumor progression, TAMs have been proposed as vehicles of tumor-targeted therapy. Nowadays, cancer immunotherapy focuses mainly on the potency of immunologic checkpoint inhibition. For example, immunotherapy with nivolumab (anti-PD-1 antibody) has been recently approved by the FDA as a second line therapeutic modality for HCC (68). However, only 20% of the HCC patients show response to therapy. Novel data demonstrated an increased efficacy of coordinated combination immunotherapy, identifying the TAMs-dependent impairment of the host adaptive immunologic competence as an attractive target (69-71).

Blockade of molecular VEGF pathways is an established angiogenic anticancer treatment option, however advantageous only in a limited number of patients. Increased tissue and serum angiopoietins associate with angiogenic switch and tumor vessel destabilization, which promotes metastasis and tumor progression (72). Inhibitors that block angiopoietin-dependent pathways decrease tumor angio- and lymphangiogenesis by interfering with TEMs and are currently in clinical development for cancer (73). Coordinated combination immunotherapy with VEGF and angiopoietin inhibition disrupts the enhanced M2 macrophage polarization in the tumor microenvironment and can boost the host anti-tumor immunity (73). In addition, a novel tumor necrosis therapy has been recently introduced (74). This approach utilizes necrosis to be a vector of curative radionuclides that accumulate in areas of necrosis.

### 1.3. Objectives

1. To inquire into the survival rates (short- and long-term) and outcome of patients with hepatobiliary tumors undergoing liver resection or LTX.
2. To identify reliable clinico-pathologic characteristics concerning the tumor biology that associate with tumor recurrence, metastasis, survival and outcome of the patients.
3. To evaluate the presence and abundance of monocyte/macrophages subpopulations, the angiopoietin axis and the formation of necrosis in the patients' tumors.
4. To assess the potency of monocyte/macrophages, angiopoietins and tumor necrosis to associate with the patient characteristics and to influence the tumor recurrence, metastasis and patient survival.
5. To investigate the potency of a coherent construct comprising monocytes/macrophages, tumor angiogenesis and necrosis to impact the clinical prognosis of patients with hepatobiliary cancer.
6. To identify patients groups with beneficial or deleterious immunologic tumor profiles that may benefit from personalized diagnostic and therapeutic strategies.

## 2. AUTHOR'S OWN WORKS

### 2.1. Clinico-experimental studies in hepatocellular carcinoma

#### 2.1.1. Lymphocytes and monocytes/macrophages impact outcome

**Atanasov G,** Dino K, Schierle K, Dietel C, Aust G, Pratschke J, Seehofer D, Schmelzle M, Hau HM. Immunologic cellular characteristics of the tumour microenvironment of hepatocellular carcinoma drive patient outcomes. *World J Surg Oncol. 2019 Jun 6;17(1):97. https://doi.org/10.1186/s12957-019-1635-3*

In hepatocarcinogenesis, the host immune system exerts a significant influence on the tumor progression. The immunologic infiltration of the tumor microenvironment with effector cells of host origin may also profoundly impact the therapeutic efficiency against cancer. Insofar, in this study, we investigated the prevalence and importance of various immunologic infiltrates invading the cancerous tissues, i.e. infiltrating monocytes/macrophages subtypes and lymphocytes (TILs), in patients (n = 58) with HCC arising de novo in the absence of liver cirrhosis. The most important findings of our work were that HCC patients with tumors characterized with high $CD68^+$ TAMs and TILs counts, revealed a substantially prolonged recurrence-free survival rates. In addition, $CD68^+$ TAMs correlated with lowered occurrence of multifocal or recurrent HCC. Conversely, M2-polarized $CD163^+$ TAMs were related to lymphangiosis carcinomatosa and multifocal disease (all $p \leq 0.05$). Taken together, our results implicate the potential implementation of monocytes/macrophages subtypes and the immunologic M2-polarization state as reliable biomarkers in patients with an advanced HCC. This might help conceptualize more personalized management tools for selected patient subgroups with beneficial or deleterious tumor characteristics. Furthermore, our findings might be useful in establishing novel adjuvant treatment options for more effective immunologic checkpoint inhibition, chemotherapy, local ablative or surgical approaches.

### 2.1.2. Angiogenic inflammation and necrosis in the tumor microenvironment influence the therapy success

**Atanasov G**, Dino K, Schierle K, Dietel C, Aust G, Pratschke J, Seehofer D, Schmelzle M, Hau HM. Angiogenic inflammation and formation of necrosis in the tumor microenvironment influence patient survival after radical surgery for de novo hepatocellular carcinoma in non-cirrhosis. *World J Surg Oncol. 2019 Dec 12;17(1):217. https://doi.org/10.1186/s12957-019-1756-8*

Complex liver surgery, either in the form of liver resection or LTX, represents the only realistic possibility for HCC patients to be cured. Liver cirrhosis, which is most commonly due to chronic alcohol consumption or viral hepatitis (HBV or HCV), provides the deleterious environment for chronic hepatic inflammation, which often culminates in HCC. However, in approximately 20% of all cases, HCC arises de novo in non-cirrhotic hepatic environment, with chronic inflammatory changes in the tumor microenvironment, different from the hepatic inflammation established in liver cirrhosis, contributing significantly for the process of hepatocarcinogenesis. Importantly, these patients present at a more progressed tumor disease and have a worse prognosis, because of no symptoms or surveillance. In the current work, we examined the abundance and importance of angiogenic TEMs, corresponding angiogenic biomarkers and the occurrence of histologic necrosis, concerning biology of HCC, survival and prognosis of the patients. Patients (n = 58) with de novo HCC in non-cirrhosis participated in the study. Noteworthy, a considerable part of the patients were diagnosed with T3/T4 advanced tumors and showed an exhibited advanced tumor biology. In this work, we were able to demonstrate that TEMs associate with an increased incidence of local and overall HCC recurrence. Moreover, TEMs correlated with an unfavorable histologic grading (moderate/poor histologic differentiation). Concerning angiopoietins, a strong correlation with metastatic and recurrent HCC could be observed, as well. In addition, necrosis associated with a higher tumor stage (T3/T4). In the current work, it associated also with elevated invasion of TEMs. Using multivariate analysis, -1 and TEMs and angiopoietins qualified as

outcome prognosticators (all $p \leq 0.05$). Therefore, we proposed a coherent construct comprising tumor necrosis, related angiogenic vehicles and cellular components as a tool foresee the prognosis of the patients after surgery for HCC. These results could deliver promising targets for immunologic checkpoint inhibition, which also incorporate a profound angiogenic potential.

### 2.1.3. Host immunologic competence associates with survival after oncologic liver transplantation

**Atanasov G**, Dino K, Schierle K, Dietel C, Aust G, Pratschke J, Seehofer D, Schmelzle M, Hau HM. Recipient hepatic tumor-associated immunologic infiltrates predict outcomes after liver transplantation for hepatocellular carcinoma. *Ann Transplant. 2020 Mar 13;25:e919414. https://doi.org/10.12659/AOT.919414*

The solid organ transplantation ultimately leads to a state of profoundly altered immunologic competence of the host. However, not only the therapeutic immunosuppressive regimes contribute to shaping the functions of the immune system after the transplantation. Even in cases of operational tolerance, in which the host co-exists with the foreign antigens of the graft without episodes of rejection, a profoundly altered immunologic responses in the host/graft universe take place. This study focused on assessing the role of angiopoietins, monocytes/macrophages subtypes and histologic tumor necrosis in the host liver prior to LTX in patients with HCC and their association with recurrence, graft rejection, survival and clinical prognosis after LTX (n = 88). Some patients were bridged prior to LTX with neoadjuvant TACE (n = 55). In our work, a coherent construct of host hepatic angiogenic factors, monocytes/macrophages and tumor necrosis was correlated with multiple tumor characteristics and patient outcome after oncologic LTX in the setting of HCC. The main findings were that tumor necrosis in patients with TACE and in both patient groups the presence of monocytes/macrophages proved to be independent prognostic biomarkers and impacted significantly patients' survival after the LTX. In patients who received bridging therapy with TACE, formation of tumor necrosis in the tumor microenvironment associated with a lowered incidence of HCC recurrence after LTX and increased frequency of monocytes/macrophages. The angiopoietin expression in the host HCC prior LTX associated with an enhanced incidence of graft rejection after the LTX. Furthermore, angiopoietin expression correlated with boosted infiltration with monocytes/macrophages (all $p \leq 0.05$). Therefore, it seems plausible that enhanced expression of recipient’s hepatic angiogenic

factors could mediate an increased graft infiltration with immune-competent, antigen-presenting monocytes/macrophages following LTX. This phenomenon could consequently trigger immunologic cascades that culminate in organ rejection.

## 2.2. Clinico-experimental studies in intrahepatic cholangiocarcinoma

### 2.2.1. Immunologic inflammation and necrosis in central area or periphery of the tumor microenvironment control outcome

**Atanasov G**, Dietel C, Feldbrügge L, Benzing C, Krenzien F, Brandl A, Mann E, Englisch JP, Schierle K, Robson SC, Splith K, Morgul MH, Reutzel-Selke A, Jonas S, Pascher A, Bahra M, Pratschke J, Schmelzle M. Tumor necrosis and infiltrating macrophages predict survival after curative resection for cholangiocarcinoma. *Oncoimmunology. 2017 Jun 28;6(8):e1331806. https://doi.org/10.1080/2162402X.2017.1331806*

Complex liver surgery represents the only realistic possibility for CCA patients to be cured. Novel data underlines the importance of distinct sites in the tumor microenvironment, such as the TCA or TIF, in regard of the tumor progression. Scientific results demonstrated that the accumulation of cellular inflammation in TCA or TIF might exert a significant impact on the patient survival. Of important note, TCA and TIF have been shown to impact the outcome in opposite manner. In ICC, the importance of the inflammation in TCA and TIF and related necrosis occurrence is unknown.

In the current work, we examined the abundance and significance of the invading TAMs in TCA or TIF, and the formation of histologic necrosis in the tumor microenvironment in regard of ICC tumor biology and recurrence, survival and prognosis of the patients. The study was conducted in 88 patients with histologically confirmed ICC and biologically naive tumors, i.e. no pretreatment with chemotherapy or radiation. Noteworthy, 53/88 (60.7%) of the patients were diagnosed with T3/T4 advanced tumors and 26/88 (29.9%) exhibited advanced tumor biology (moderate/poor histologic differentiation). In this work, we were able to demonstrate that $CD68^+$ TAMs associate with a reduced incidence of tumor recurrence. In addition, low TAMs counts correlated with unfavorable histologic grading (moderate/poor histologic differentiation) and increased incidence of lymphangiosis carcinomatosa. In addition, tumor necrosis, associated with a more frequent angioinvasion and formation of multiple ICC nodules. Moreover, in the current work, we were able to demonstrate that the immunologic

infiltration of TCA or TIF with monocytes/macrophages influences patient survival and outcome in a different manner. Using multivariate analysis, TAMs and necrosis were also demonstrated to be independent prognosticators concerning patient survival (all $p \leq 0.05$). Our study reveals the complex nature of human CCA and delineates the important roles of distinct immunologic sites in the vicinity of the tumor, i.e. TCA and TIF, concerning tumor progression and patient survival. Therefore, we suggest the immunologic coherence comprised of necrosis and TAMs, ant their further relation to TCA or TIF, as a refined diagnostic device to read the outcome of patients undergoing major hepatectomy for ICC.

## 2.3. Clinico-experimental studies in perihilar cholangiocarcinoma

### 2.3.1. Tumor-associated macrophages affect survival rates

**Atanasov G*,** Hau HM*, Dietel C, Benzing C, Krenzien F, Brandl A, Wiltberger G, Matia I, Prager I, Schierle K, Robson SC, Reutzel-Selke A, Pratschke J, Schmelzle M, Jonas S. Prognostic significance of macrophage invasion in hilar cholangiocarcinoma. *BMC Cancer 2015;15:790. https://doi.org/10.1186/s12885-015-1795-7* *equal contribution

PHC translates into detrimental clinical prognosis and outcome. The risk of PHC recurrence remains a major obstacle in this scenario, even if hilar en bloc resection is added into the surgical approach. Novel and reliable prognostic biomarkers related to tumor recurrence and patient survival in this rare and devastating disease are urgently needed. TAMs clinical relevance in PHC is unknown. Therefore, in the current work we assessed the presumed relationships of monocytes/macrophages invasion and metastatic or recurrent disease, survival and prognosis in patients suffering from PHC. We included in the study 47 cases who received liver resection with curative intent. Neoadjuvant chemotherapy or radiation were not applied, thus providing unaltered tumor biology for our immunological studies. In 25/47 (53.2%) patients an overall tumor recurrence was detected. In other 20/47 (42.6%) cases a local recurrence was diagnosed. In this work, we were able to demonstrate that the presence of $CD68^+$ TAMs was strongly related to a significantly more frequent PHC recurrence. Moreover, $CD68^+$ TAMs also affected significantly patient overall and recurrence-free survival after surgery: patients with tumors infiltrated with high frequencies of TAMs revealed a significantly deteriorated survival rates. The presence of TAMs and recurrent PHC were demonstrated to be independent predictive biomarkers in the multivariate analysis (all $p \leq 0.05$). Therefore, PHC invasion with monocytes/macrophages may represent a crucial step in the etiology and pathogenesis of this tumor entity, and their diagnostic utilization or the therapeutic targeting of TAMs may deliver novel management options.

### 2.3.2. TIE2-expressing monocytes and related angiopoietin axis utilize personalized outcome stratification

**Atanasov G,** Hau HM, Dietel C, Benzing C, Krenzien F, Brandl A, Wiltberger G, Schierle K, Robson SC, Reutzel-Selke A, Pascher A, Jonas S, Pratschke J, Schmelzle M. Prognostic Significance of TIE2-Expressing Monocytes in Hilar Cholangiocarcinoma. *Journal of Surgical Oncology 2016;114: 91-98. https://doi.org/10.1002/jso.24249*

Tumor-related factors of angiogenesis and corresponding receptor-bearing immunologic infiltrates may represent novel biomarkers that identify subgroups of patients, who may profit from individualized standards of care. In this setting, angiopoietin-1 and angiopoietin-2 represent key growth factors and regulators of tumor angiogenesis. Of note, concerning their function, angiopoietin-1 and angiopoietin-2 have been shown to act as antagonists with the ability to activate the angiopoietin receptor TIE2 on invading monocytes. The importance of these angiogenic factors and related TEMs has been documented for several human cancer types, where angiogenesis is known to exert a key influence on cancerogenesis. However, their role in poorly vascularized PHC is unknown. Therefore, in the current work we aimed to assess their presence in PHC. In a next step, we aimed to investigate their clinical significance concerning metastasis, recurrence, survival and prognosis in PHC patients (n = 47). High density of tumor angiopoietin-1 was associated with a significantly decreased incidence of metastatic PHC. On the other side, TEMs were correlated to a substantially mitigated recurrent PHC. Presence of TEMs was correlated with a marked improvement of the PHC patients' survival (overall and recurrence-free rates). For instance, the overall survival was 83.2%, 62.2%, and 56.6%, when compared to 71.3%, 36.3%, and 14.9% in regard of presence or absence of TEMs, respectively. In the multivariate analysis, TEMs and PHC recurrence could be identified as independent prognosticators (all $p \leq 0.05$). In the current work, we were able to demonstrate for the first time that angiogenic TEMs are present in the tumors of PHC patients. Moreover, we showed that these immunologic

effectors, together with the related angiopoietin axis, play a crucial role in PHC and, thus, offer the opportunity to define a patient subgroup with a beneficial tumor biology.

### 2.3.3. Histologic tumor necrosis defines clinical prognosis

**Atanasov G,** Schierle K, Hau HM, Dietel C, Krenzien F, Brandl A, Wiltberger G, Englisch, J, Robson SC, Reutzel-Selke A, Pascher A, Jonas S, Pratschke J, Christian Benzing, Moritz Schmelzle. Prognostic significance of tumor necrosis in hilar cholangiocarcinoma. *Ann Surg Oncol 2017 Feb; 24(2):518-525. https://doi.org/10.1245/s10434-016-5472-0*

In this work, to the best of our knowledge, our group was the first to demonstrate that necrosis in the tumor microenvironment of PHC is significantly related to patients' survival and clinical prognosis after curative therapy. The curative therapeutic approaches for PHC comprise aggressive surgical strategies often including extrahepatic bile duct and portal vein resection and reconstruction. However, only marginal improvements in the multimodal patient management and success of therapy outcome could be achieved in recent times. Therefore, the insights in the biology of PHC will help to identify biomarkers concerning clinical prognosis and this, in turn, will help to establish individualized management concepts. The importance of tumor necrosis in PHC is unknown. Of note, necrotic tumor tissue might well characterize subsets of PHC, based on the fact that PHC are mostly hypovascular tumors with a high amount of hypoxic cells. In this work (n = 47) we aimed to explore the importance of the formation of necrosis in the tumor microenvironment of PHC. Twenty-eight out of 47 (59.6%) PHC patients showed no tumor necrosis. On the other side, histologic tumor necrosis was apparent in 19/47 (40.4%) PHC patients. In this subgroup, 15/19 (79%) cases displayed only mild and 4/19 (21%) cases exhibited manifestation of severe necrosis. Our results demonstrated that formation of necrosis affected patient survival. In patients with necrosis, the overall and recurrence-free survival rates were substantially decreased. Moreover, in the subgroup with formation of mild or severe necrosis a significantly deteriorated survival was apparent, as well. In the multivariate analysis, PHC histological differentiation and tumor necrosis proved to be independent biomarkers (all $p < 0.05$). Management and prognosis stratification in PHC mostly depend on histopathological evaluation. The identification of the prognostic value of the formation of necrosis in the tumor

microenvironment of PHC defines an attractive and easily assessable target. This asset might help to optimize the individualized risk stratification for cases with increased risk for poor outcome, in whom adjuvant therapeutic options are warranted.

## 3. DISCUSSION

The significance of the host immune system and its potency in facilitating anti-cancer responses has been vastly underestimated in the past. Only in recent years, the major role of multiple immunologic effectors has been recognized and taken into consideration regarding improvement of diagnostic and therapeutic approaches, and disease prognosis. Novel research delivered astonishing results demonstrating that long-term survival is not illusory for patients with advanced and highly aggressive metastatic cancers, if the host immunologic competence is properly exploited (75, 76). In addition, combination regimen comprising established chemotherapy protocols and additional blockade of immunologic pathways with so called checkpoint inhibitors, delivered promising data in regard of tumor recurrence, survival and prognosis (77). Insofar, in the current research, focus was set on the various arms of the host immunologic effectors, related angiogenic biomarkers and tumor necrosis in regard to presumed efficacy in defining disease prognosis, predicting patient' survival and associating with established markers of cancer aggressiveness, i.e. tumor recurrence.

The worldwide incidence of hepatobiliary cancer is increasing. The pronounced aggressiveness and outlined ability to progress to a disseminated, metastatic disease, translating into no responsiveness to chemotherapy or eligibility for curative surgery, represent major obstacles on a clinical daily basis. Rapid progression of fulminant liver failure, accompanied by the obstruction of the biliary tree and septic complications deprive these patients of much time left after diagnosis. In addition, even after curative therapy is accomplished in the limited numbers of patients, the recurrence rates in the further course are immense, contributing essentially to the worldwide high mortality.

In the presented works, we were able to demonstrate that in hepatobiliary cancer various monocytes/macrophages subpopulations significantly influence patient' survival rates and associate not only with local and overall recurrence, but also with a wide range of established predictors of cancer aggressiveness and patient outcome, i.e. histologic grading, lymph node involvement, metastasis, perineural and lymphovascular invasion, and T stage. Moreover,

the assessment of the occurrence of histologic necrosis in the tumor microenvironment of the various hepatobiliary malignancies revealed similar data and demonstrated prognostic significance in the multivariate analysis. Taken together, our data delivered evidence that an immunological construct comprising of necrosis, monocytes/macrophages and associated angiogenic biomarkers in the tumor microenvironment is coherently established in hepatobiliary malignancies and demonstrates a marked potency to impact survival and recurrence rates, and clinical prognosis.

In our works, monocytes/macrophages influenced patient survival. However, depending on the type of biliary cancer, effects on survival can be opposite. This duality represent an established phenomenon that is well documented in the literature (78-82). Several explanations can shed light on this. Monocyte/macrophage functions can be steered by the establishment of hypoxic conditions and tumor necrosis in the microenvironment of cancerous tissues. Partial pressure difference in oxygen concentrations varies between the center and periphery of cancer. This exerts influence on monocyte/macrophage polarization state depending on their localization in these distinct tumor sites/locations, i.e. in TCA or TIF, respectively (84). In this setting, altered levels of mediators related to hypoxia, like hypoxia-inducible factor 1/2-α (HIF1/2-α), may drive the polarization state into M1 or M2 mode (83-85).

Another possible explanation is that monocytes/macrophages may exert opposite impact on survival depending on the underlying tumor biology, which might be profoundly different in various cancer types, i.e. classic Hodgkin lymphoma and colorectal cancer (86-88). Furthermore, the extent of tumor vascularization may also essentially impact the polarization state of infiltrating monocytes/macrophages. For example, in our works, TEMs associate with negative survival in HCC, which conforms to the data published in the literature so far (89-91). The negative influence on survival rates is documented for malignancies with a strong neoangiogenesis, where tumor progression is highly dependent on the formation of an

extended neovascularization (92). In these scenarios, TEMs have been shown to foster tumor-related angiogenic pathways via the TIE2/angiopoietin-2-axis (52, 54, 55).

However, in PHC the impact on survival was beneficial. In this tumor entity, the extent of neovascularization is mostly tenuous and this implies that alternative monocyte functions might be deployed, which can ultimately restrain the tumor progression. Furthermore, recent results revealed that angiopoietin 1- and -2 act as functional antagonists highlighting beneficial effects of angiopoietin-1 signaling in human cancer (93, 94). In addition, novel data linked the enhanced angiogenic microRNAs signaling and protective angiopoietin-1 direct effects in CCA to an intensified homing of TEMs in tumors, which exerted a negative influence on CCA progression (95). Moreover, our results are also in line with published data that outlined TEMs functions to be mechanistically involved in liver regeneration and to positively influence the outcome of the patients (96-98).

Another important remark on the bimodal functionality of monocytes/macrophages concerning tumor progression, patients' survival and prognosis is that the established classification into M1 and M2 types is mainly schematic and might mirror only the tip of the iceberg. This means, that taking into consideration of only one cellular marker to reliably describe the pronounced functional characteristics of immune cells, i.e. the TIE2 receptor on TEMs, or CD68 on TAMs, is insufficient. Therefore, regarding future therapeutic implications and diagnostic improvements, a further research and identification of key molecular pathways and related biomarkers is warranted.

Monocytes/macrophages orchestrate the host immune responses that can significantly influence the effectiveness of conventional chemotherapy regimen. In the presented works, we were able to identify various subsets of tumor-infiltrating monocytes/macrophages, tumor angiogenesis and formation of tumor necrosis as reliable prognosticators of patient survival and outcome. The implementation of so-called 'first-generation' immunomodulatory agents against Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) or PD-L1 has revolutionized the landscape of treatment options for some advanced cancers (99). Targeting of alternative

molecular pathways other than core inhibitory modules may exert a major influence on the host antitumor immune competence, as well. Therefore, 'next generation' immunomodulatory agents represent a major frontier in translational research and a realistic hope in the bench to bedside paradigm.

In the context of adjunct angiogenic therapy for advanced hepatobiliary cancer, the limitations of anti-VEGF treatment have come to the fore in recent years. The presented studies may deliver important insights in regard of this scenario. In addition, emerging experimental research highlighted the ability of monocyte/macrophages to limit the efficacy of the anti-VEGF treatment. Treatment with bevacizumab translated into an increased TAMs infiltration in the vascular cancer vicinity via CX3CL1-dependent pathways, which in turn inhibited the host antitumor immunity, deploying IL10-dependent mechanisms (100). Of note, monocytes/macrophage depletion resulted into marked improvements of anti-VEGF treatment and antitumor immunity. Moreover, Park et al demonstrated that combination therapy with TIE2-pathway activation under a concomitant blockade of angiopoietin-2 leads to a major decrease in tumor progression (101). Scientific background of these results is the implementation of the hypothesis that antiangiogenic treatment with VEGF facilitates hypoxic conditions in the tumor microenvironment, ultimately leading to an activation of tumor-escape mechanisms, which translate into an increased expression of angiopoietin-2 and intensified infiltration with monocytes/macrophages. Taken together, these conditions foster tumor angiogenesis and progression. Therefore, restoring the immunologic homeostasis utilizing TIE2-pathway activation / inhibition of angiopoietin-2 could mediate sufficient tissue oxygen delivery but hamper excessive tumor angiogenesis and my represent a novel molecular pathway for immunologic checkpoint inhibition.

Data on the importance of the occurrence of necrosis in the tumor microenvironment is rare. However, recent research highlighted that the formation of necrosis is associated with hypoxia and the activation of host immunologic responses in the tumor microenvironment that lead to increased accumulation with immune-competent effectors and angiogenic switch.

In the scope of immune escape, this facilitates tumor progression and poor prognosis (102, 103). In the presented works, the occurrence of necrosis in the tumor microenvironment was significantly related to poor outcome. A promising 'from bench to bedside translation' of these results can be, apart from the routine implementation for individualized histopathological risk/prognosis stratification, the newly introduced tumor necrosis therapy (TNT) (104, 105). This novel approach provides a promising therapeutic option for advanced cancer. Utilizing radiolabeled agents demonstrating an established therapeutic efficacy in necrotic tissues, the occurrence of necrosis in the tumor microenvironment can be taken advantage of as a vector of therapeutic radionuclides.

The presented data delivered insights also on the importance of the host hepatic immunologic load and related factors of angiogenesis concerning the course following oncologic LTX. Our study showed that a coherent construct of host hepatic angiogenic factors, monocytes/macrophages and tumor necrosis associates with multiple tumor characteristics and patient outcome after oncologic LTX. In addition, tumor necrosis in patients with TACE and in both patient groups the presence of monocytes/macrophages qualified as independent prognostic biomarkers and impacted significantly survival after the LTX. In patients who received bridging therapy with TACE, tumor necrosis correlated with a decreased incidence of recurrent HCC after LTX and intensified infiltration with monocytes/macrophages.

LTX is an established lifesaving and curative approach. However, graft rejection exerts a negative impact on clinical prognosis and baseline immunosuppressive regimen are deployed for the entire course after LTX (106). Patient and graft survival have been substantially improved with the implementation of potent anti-rejection agents (107). However, the long-term outcome of patient and graft survival remains unsatisfactory due to the complications that are largely associated with lifelong immunosuppression. Therefore, strategies for intentional operational tolerance have been recently introduced (108, 109). Moreover, in the setting of oncologic LTX for HCC, establishment of operational tolerance

has the potential to mitigate not only the long-term adverse effects of immunosuppression, but also to decrease the risk of cancer related to it.

Recent studies demonstrated the clinical feasibility of operational tolerance in the setting of oncologic LTX (110). Interestingly, our study revealed that angiopoietin expression in the host HCC prior LTX associated with increased episodes of graft rejection after the LTX. In addition, angiopoietin expression associated with elevated infiltration with antigen-presenting monocytes/macrophages, as well. Data on the importance of the host immunologic competence on the course after transplantation are scarce. However, it seems possible that host-related hepatic immunologic characteristics can have a dramatic influence on the extent of immunologic surveillance and rejection episodes after LTX. Thus, the presented study delivered results that help to define subgroups of patients that are at high risk for organ rejection after LTX. The presented data may also find future utilization in the setting of operational tolerance in providing selection criteria for HCC liver transplant recipients to be eligible for clinical trials of operational tolerance.

As stated in chapter 1.3., the main objectives of the current works were to finally explore the potential of the presumed immunologic coherence comprising monocytes/macrophages, tumor angiogenesis and necrosis to prognosticate the clinical outcome in patients with hepatobiliary cancer and thereby identify subgroups of patients with immunologic tumor profiles that may benefit from personalized management. Our findings demonstrated necrosis associated with monocytes/macrophages and angiopoietins. Furthermore, this construct consisting of tumor necrosis, TAMs and angiopoietin associated with recurrent disease and patient' survival following resection or LTX, delineating its prognostic significance. However, there is only limited data about the mechanistic links that involve the angiopoietin axis, monocytes/macrophages and necrosis occurrence in carcinogenesis. Therefore, a further basic research will deliver important hints in conceptualizing novel approaches of immunologic checkpoint inhibition for hepatobiliary malignancies.

## 4. SUMMARY

The incidence of hepatobiliary cancer has increased worldwide in recent years. These tumor entities translate into high recurrence and mortality rates, poor prognosis and clinical outcome. The management of hepatobiliary cancer is challenging and demands in most cases referral to a specialized high volume center where meticulous therapeutic interventions and advanced surgery can be performed. Unfortunately, only limited therapeutic modalities are available and curative surgery or LTX are often precluded due to the advanced disease at time of diagnosis. In this palliative setting, chemotherapy, radiation or best supportive care remain the only pillars of medical care.

There is an urgent unmet need for adjunct therapeutic modalities for hepatobiliary cancer. Hereby, the role of the host immune system, after falling into desuetude in the past, has been nowadays increasingly recognized as a powerful tool in the armamentarium of available options. Overwhelming data demonstrates the formidable ability of various entities of the innate and adaptive immune system not only to be efficiently applied as vectors of anticancer treatment, but also help refine the diagnostic strategies. The latter translates into improved risk stratification and enhanced ability to identify subgroups of patients with negative tumor profiles and poor prognosis. Taken together, these insights can help enhance the efficacy of cancer management and deliver more individualized approaches in an era of personalized medicine.

In the current studies, we were able to demonstrate the prognostic significance of various aspects of the host immune system and establish links that presume functional networks. This enabled us to outline them as an autonomous immunologic construct in the setting of human cancer. The main conclusions were that tumor-infiltrating monocytes/macrophages subtypes, related angiogenesis vectors and the formation of histologic necrosis in the tumor microenvironment are strongly associated. This coherent immunologic construct exerted a significant influence on patients' overall and recurrence-free survival and qualified as an independent marker of predictiveness. The presented studies generated data that could help

efficiently categorize patients in subgroups of increased risk for unfavorable clinical outcome. Furthermore, these results may represent a novel molecular pathway for immunologic checkpoint inhibition in human hepatobiliary cancer. Future studies are needed to better explore and define the implied mechanistic links, which will not only facilitate a better understanding of the tumor biology, but also optimize the management of cancer.

## 5. REFERENCES


1. Pang TC, Lam VW. Surgical management of hepatocellular carcinoma. World J Hepatol. 2015 Feb 27;7(2):245-52. doi: 10.4254/wjh.v7.i2.245.
2. Maithel SK, Gamblin TC, Kamel I, Corona-Villalobos CP, Thomas M, Pawlik TM. Multidisciplinary approaches to intrahepatic cholangiocarcinoma. Cancer. 2013 Nov 15;119(22):3929-42. doi: 10.1002/cncr.28312.
3. Mansour JC, Aloia TA, Crane CH, Heimbach JK, Nagino M, Vauthey JN. Hilar cholangiocarcinoma: expert consensus statement. HPB (Oxford). 2015 Aug;17(8):691-9. doi: 10.1111/hpb.12450.
4. Lurje I, Czigany Z, Bednarsch J, Roderburg C, Isfort P, Neumann UP, Lurje G. Treatment Strategies for Hepatocellular Carcinoma - a Multidisciplinary Approach. Int J Mol Sci. 2019 Mar 22;20(6). pii: E1465. doi: 10.3390/ijms20061465.
5. Mody K, Abou-Alfa GK. Systemic Therapy for Advanced Hepatocellular Carcinoma in an Evolving Landscape. Curr Treat Options Oncol. 2019 Jan 11;20(2):3. doi: 10.1007/s11864-019-0601-1.
6. Ebata T, Hirano S, Konishi M, Uesaka K, Tsuchiya Y, Ohtsuka M, Kaneoka Y, Yamamoto M, Ambo Y, Shimizu Y, Ozawa F, Fukutomi A, Ando M, Nimura Y, Nagino M; Bile Duct Cancer Adjuvant Trial (BCAT) Study Group. Randomized clinical trial of adjuvant gemcitabine chemotherapy versus observation in resected bile duct cancer. Br J Surg. 2018 Feb;105(3):192-202. doi: 10.1002/bjs.10776.
7. Killock D. Immunotherapy: Nivolumab keeps HCC in check and opens avenues for checkmate. Nat Rev Clin Oncol. 2017 Jul;14(7):392. doi: 10.1038/nrclinonc.2017.70.
8. Kantoff PW, Higano CS, Shore ND, et al: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-422.
9. Hodi FS, O'Day SJ, McDermott DF, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-223.
10. Robert C, Thomas L, Bondarenko I, et al: Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364:2517-2526.
11. Ozer Etik D, Suna N, Boyacioglu AS. Management of Hepatocellular Carcinoma: Prevention, Surveillance, Diagnosis, and Staging. Exp Clin Transplant. 2017 Mar;15(Suppl 2):31-35.
12. Chunlertrith K, Sukeepaisarnjaroen W, Mairiang P, Urwijitaroon Y, Takase K, Yamauchi T, Yoshimura H, Tameda Y. Clinico-epidemiology of hepatitis C viral infection in northeastern Thailand. Southeast Asian J Trop Med Public Health. 2000 Jun;31(2):273-6.
13. Liang T, Chen EQ, Tang H. Hepatitis B virus gene mutations and hepatocarcinogenesis. Asian Pac J Cancer Prev. 2013;14(8):4509-13.

14. Wiangnon S, Kamsa-ard S, Suwanrungruang K, Promthet S, Kamsa-ard S, Mahaweerawat S, Khuntikeo N. Trends in incidence of hepatocellular carcinoma, 1990-2009, Khon Kaen, Thailand. Asian Pac J Cancer Prev. 2012;13(3):1065-8.
15. Mohamad B, Shah V, Onyshchenko M, Elshamy M, Aucejo F, Lopez R, Hanouneh IA, Alhaddad R, Alkhouri N. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. Hepatol Int. 2016 Jul;10(4):632-9. doi: 10.1007/s12072-015-9679-0.
16. Somboon K, Siramolpiwat S, Vilaichone RK. Epidemiology and survival of hepatocellular carcinoma in the central region of Thailand. Asian Pac J Cancer Prev. 2014;15(8):3567-70.
17. Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011 Mar;53(3):1020-2. doi: 10.1002/hep.24199
18. Lee KK, Kim DG, Moon IS, Lee MD, Park JH. Liver transplantation versus liver resection for the treatment of hepatocellular carcinoma. J Surg Oncol. 2010 Jan 1;101(1):47-53. doi: 10.1002/jso.21415.
19. Yao FY, Bass NM, Nikolai B, Davern TJ, Kerlan R, Wu V, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: analysis of survival according to the intention-to-treat principle and dropout from the waiting list. Liver Transpl. 2002 Oct;8(10):873-83.
20. Qu Z, Ling Q, Gwiasda J, Xu X, Schrem H, Beneke J, Kaltenborn A, Krauth C, Mix H, Klempnauer J, Emmanouilidis N. Hangzhou criteria are more accurate than Milan criteria in predicting long-term survival after liver transplantation for HCC in Germany. Langenbecks Arch Surg. 2018 Aug;403(5):643-654. doi: 10.1007/s00423-018-1696-8.
21. Crissien AM, Frenette C. Current management of hepatocellular carcinoma. Gastroenterol Hepatol (N Y). 2014 Mar;10(3):153-61.
22. Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011 Mar;53(3):1020-2. doi: 10.1002/hep.24199.
23. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008 Jul 24;359(4):378-90. doi: 10.1056/NEJMoa0708857.

24. Casadei Gardini A, Foca F, Scartozzi M, Silvestris N, Tamburini E, Faloppi L, Brunetti O, Rudnas B, Pisconti S, Valgiusti M, Marisi G, Foschi FG, Ercolani G, Tassinari D, Cascinu S, Frassineti GL. Metronomic capecitabine versus best supportive care as second-line treatment in hepatocellular carcinoma: a retrospective study. Sci Rep. 2017 Feb 13;7:42499. doi: 10.1038/srep42499.
25. Chinchilla-López P, Aguilar-Olivos NE, García-Gómez J, Hernández-Alejandro KK, Chablé-Montero F, Motola-Kuba D, Patel T, Méndez-Sánchez N. Prevalence, Risk Factors, and Survival of Patients with Intrahepatic Cholangiocarcinoma. Ann Hepatol. 2017 Jul-Aug;16(4):565-568. doi: 10.5604/01.3001.0010.0293.
26. Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet. 2014 Jun 21;383(9935):2168-79. doi: 10.1016/S0140-6736(13)61903-0
27. DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg. 2007 May;245(5):755-62.
28. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, D'Angelica M, DeMatteo RP, Fong Y, Schwartz L, Kemeny N, O'Reilly E, Abou-Alfa GK, Shimada H, Blumgart LH, Jarnagin WR. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. Ann Surg. 2008 Jul;248(1):84-96. doi: 10.1097/SLA.0b013e318176c4d3.
29. Li YY, Li H, Lv P, Liu G, Li XR, Tian BN, Chen DJ. Prognostic value of cirrhosis for intrahepatic cholangiocarcinoma after surgical treatment. J Gastrointest Surg. 2011 Apr;15(4):608-13. doi: 10.1007/s11605-011-1419-8.
30. Sapisochin G, Fidelman N, Roberts JP, Yao FY. Mixed hepatocellular cholangiocarcinoma and intrahepatic cholangiocarcinoma in patientsundergoing transplantation for hepatocellular carcinoma. Liver Transpl. 2011 Aug;17(8):934-42. doi: 10.1002/lt.22307
31. Kiefer MV, Albert M, McNally M, Robertson M, Sun W, Fraker D, Olthoff K, Christians K, Pappas S, Rilling W, Soulen MC. Chemoembolization of intrahepatic cholangiocarcinoma with cisplatinum, doxorubicin, mitomycin C, ethiodol, and polyvinyl alcohol: a 2-center study. Cancer. 2011 Apr 1;117(7):1498-505. doi: 10.1002/cncr.25625.
32. Park SY, Kim JH, Yoon HJ, Lee IS, Yoon HK, Kim KP. Transarterial chemoembolization versus supportive therapy in the palliative treatment of unresectable intrahepatic cholangiocarcinoma. Clin Radiol. 2011;66:322–28.
33. Vogl TJ, Naguib NN, Nour-Eldin NE, et al. Transarterial chemoembolization in the treatment of patients with unresectable cholangiocarcinoma: results and prognostic factors governing treatment success. Int J Cancer. 2012;131:733–40.

34. Kuhlmann JB, Euringer W, Spangenberg HC, et al. Treatment of unresectable cholangiocarcinoma: conventional transarterial chemoembolization compared with drug eluting bead-transarterial chemoembolization and systemic chemotherapy. Eur J Gastroenterol Hepatol. 2012;24:437–43.
35. Valle J, Wasan H, Palmer DH, et al. ABC-02 Trial Investigators Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362:1273–81.
36. Deoliveira ML, Schulick RD, Nimura Y, Rosen C, Gores G, Neuhaus P, Clavien PA. New staging system and a registry for perihilar cholangiocarcinoma. Hepatology. 2011 Apr; 53(4):1363-71.
37. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, Fichtner-Feigl S, Lorf T, Goralcyk A, Hörbelt R, Kroemer A, Loss M, Rümmele P, Scherer MN, Padberg W, Königsrainer A, Lang H, Obed A, Schlitt HJ. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg. 2012 Mar; 255(3):405-14.
38. Ribero D, Pinna AD, Guglielmi A, et al. Surgical approach for long-term survival of patients with intrahepatic cholangiocarcinoma: a multi-institutional analysis of 434 patients. Arch Surg. 2012;147:1107–1113. doi: 10.1001/archsurg.2012.1962.
39. Lee SG, Song GW, Hwang S, et al. Surgical treatment of hilar cholangiocarcinoma in the new era: the Asan experience. J Hepatobiliary Pancreat Sci. 2010;17:476–489. doi: 10.1007/s00534-009-0204-5.
40. Rosen CB, Heimbach JK, Gores GJ. Liver transplantation for cholangiocarcinoma. Transpl Int. 2010 Jul; 23(7):692-7.
41. Darwish Murad S, Kim WR, Therneau T, Gores GJ, Rosen CB, Martenson JA, Alberts SR, Heimbach JK. Predictors of pretransplant dropout and posttransplant recurrence in patients with perihilar cholangiocarcinoma. Hepatology. 2012 Sep; 56(3):972-81.
42. Lee J, Park SH, Chang HM, Kim JS, Choi HJ, Lee MA, Jang JS, Jeung HC, Kang JH, Lee HW, Shin DB, Kang HJ, Sun JM, Park JO, Park YS, Kang WK, Lim HY. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2012 Feb; 13(2):181-8
43. Geynisman DM, Catenacci DV. Toward personalized treatment of advanced biliary tract cancers. Discov Med. 2012 Jul; 14(74):41-57.
44. Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. Oncogene. 2013 Oct 10; 32(41):4861-70.

45. Brivio S, Cadamuro M, Strazzabosco M, Fabris L. Tumor reactive stroma in cholangiocarcinoma: The fuel behind cancer aggressiveness. World J Hepatol. 2017 Mar 28; 9(9):455-468.
46. Degroote H, Van Dierendonck A, Geerts A, Van Vlierberghe H, Devisscher L. Preclinical and Clinical Therapeutic Strategies Affecting Tumor-Associated Macrophages in Hepatocellular Carcinoma. J Immunol Res. 2018 Oct 16;2018:7819520. doi: 10.1155/2018/7819520.
47. Goswami KK, Ghosh T, Ghosh S, Sarkar M, Bose A, Baral R. Tumor promoting role of anti-tumor macrophages in tumor microenvironment. Cell Immunol. 2017 Jun;316:1-10. doi: 10.1016/j.cellimm.2017.04.005.
48. Capece D, Fischietti M, Verzella D, Gaggiano A, Cicciarelli G, Tessitore A, Zazzeroni F, Alesse E. The inflammatory microenvironment in hepatocellular carcinoma: a pivotal role for tumor-associated macrophages. Biomed Res Int. 2013;2013:187204. doi: 10.1155/2013/187204.
49. Shirabe K, Mano Y, Muto J, Matono R, Motomura T, Toshima T, Takeishi K, Uchiyama H, Yoshizumi T, Taketomi A, Morita M, Tsujitani S, Sakaguchi Y, Maehara Y. Role of tumor-associated macrophages in the progression of hepatocellular carcinoma. Surg Today. 2012 Jan;42(1):1-7. doi: 10.1007/s00595-011-0058-8.
50. Petty AJ, Yang Y. Tumor-associated macrophages: implications in cancer immunotherapy. Immunotherapy. 2017 Mar;9(3):289-302. doi: 10.2217/imt-2016-0135.
51. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology. 2013 Mar;144(3):512-27. doi: 10.1053/j.gastro.2013.01.002.
52. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, Sampaolesi M, Naldini L. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell. 2005 Sep; 8(3):211-26.
53. Asahara T, Chen D, Takahashi T, Fujikawa K, Kearney M, Magner M, Yancopoulos GD, Isner JM. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. Circ Res. 1998 Aug 10; 83(3):233-40.
54. Venneri MA, De Palma M, Ponzoni M, Pucci F, Scielzo C, Zonari E, Mazzieri R, Doglioni C, Naldini L. Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. Blood. 2007 Jun 15; 109(12):5276-85.
55. Bron S, Henry L, Faes-Van't Hull E, Turrini R, Vanhecke D, Guex N, Ifticene-Treboux A, Marina Iancu E, Semilietof A, Rufer N, Lehr HA, Xenarios I, Coukos G, Delaloye JF, Doucey MA. TIE-2-expressing monocytes are lymphangiogenic and associate

specifically with lymphatics of human breast cancer. Oncoimmunology. 2016 Feb; 5(2):e1073882.

56. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011 May 19;473(7347):298-307. doi:10.1038/nature10144.
57. Park JH, Choi H, Kim YB, Kim YS, Sheen SS, Choi JH, Lee HL, Lee KS, Chung WY, Lee S, Park KJ, Hwang SC, Lee KB, Park KJ. Serum angiopoietin-1 as a prognostic marker in resected early stage lung cancer. Lung Cancer. 2009 Dec;66(3):359-64. doi: 10.1016/j.lungcan.2009.03.002.
58. Park JH, Park KJ, Kim YS, Sheen SS, Lee KS, Lee HN, Oh YJ, Hwang SC. Serum angiopoietin-2 as a clinical marker for lung cancer. Chest. 2007 Jul;132(1):200-6. Epub 2007 May 15.
59. Torimura T, Ueno T, Kin M, Harada R, Taniguchi E, Nakamura T, Sakata R, Hashimoto O, Sakamoto M, Kumashiro R, Sata M, Nakashima O, Yano H, Kojiro M. Overexpression of angiopoietin-1 and angiopoietin-2 in hepatocellular carcinoma. J Hepatol. 2004 May;40(5):799-807.
60. Fagiani E, Christofori G. Angiopoietins in angiogenesis. Cancer Lett. 2013 Jan 1;328(1):18-26. doi: 10.1016/j.canlet.2012.08.018.
61. Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol. 2009 Mar;10(3):165-77. doi: 10.1038/nrm2639.
62. Chen L, Zeng X, Kleibeuker E, Buffa F, Barberis A, Leek RD, Roxanis I, Zhang W, Worth A, Beech JS, Harris AL, Cai S. Paracrine effect of GTP cyclohydrolase and angiopoietin-1 interaction in stromal fibroblasts on tumor Tie2 activation and breast cancer growth. Oncotarget. 2016 Feb 23;7(8):9353-67. doi: 10.18632/oncotarget.6981
63. Seki E, Schwabe RF. Hepatic inflammation and fibrosis: Functional links and key pathways. Hepatology 2015; 61:1066-79; PMID:25066777; https://doi.org/10.1002/hep.27332
64. Liu X, Qiu H, Zhang P, Feng X, Chen T, Li Y, Tao K, Li G, Sun X, Zhou Z; China GastrointestinalStromal Tumor Study Group (CN-GIST). Prognostic role of tumor necrosis in patients undergoing curative resection for gastric gastrointestinal stromal tumor: a multicenter analysis of 740 cases in China. Cancer Med. 2017 Dec;6(12):2796-2803. doi: 10.1002/cam4.1229.
65. Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 2014; 60:1090-6;
66. Bredholt G, Mannelqvist M, Stefansson IM, Birkeland E, Bø TH, Øyan AM, Trovik J, Kalland KH, Jonassen I, Salvesen HB, Wik E, Akslen LA. Tumor necrosis is an

important hallmark of aggressive endometrial cancer and associates with hypoxia, angiogenesis and inflammation responses. Oncotarget. 2015 Nov 24;6(37):39676-91. doi: 10.18632/oncotarget.5344.

67. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol. 2004 Aug;4(8):641-8. doi: 10.1038/nri1415.
68. European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. Journal of Hepatology. 2018;69(1):182–236.
69. Simpson T. R., Li F., Montalvo-Ortiz W., et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti–CTLA-4 therapy against melanoma. Journal of Experimental Medicine. 2013;210(9):1695–1710. doi: 10.1084/jem.20130579.
70. Romano E., Kusio-Kobialka M., Foukas P. G., et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(19):6140–6145. doi: 10.1073/pnas.1417320112
71. Zhu Y., Knolhoff B. L., Meyer M. A., et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Research. 2014;74(18):5057–5069. doi: 10.1158/0008-5472.CAN-13-3723.
72. Schmittnaegel M, De Palma M. Reprogramming Tumor Blood Vessels for Enhancing Immunotherapy. Trends Cancer. 2017 Dec;3(12):809-812. doi: 10.1016/j.trecan.2017.10.002. Epub 2017 Oct 27
73. Saharinen P, Eklund L, Alitalo K. Therapeutic targeting of the angiopoietin-TIE pathway. Nat Rev Drug Discov. 2017 Sep;16(9):635-661. doi: 10.1038/nrd.2016.278.
74. Cona MM, Wang H, Li J, Feng Y, Chen F, de Witte P, Verbruggen A, Ni Y. Continuing pursuit for ideal systemic anticancer radiotherapeutics. Invest New Drugs. 2012 Oct;30(5):2050-65. doi: 10.1007/s10637-011-9758-6.
75. Higuchi R, Yamamoto M, Hatori T, Shimizu K, Imai K, Takasaki K. Intrahepatic cholangiocarcinoma with lymph node metastasis successfully treated by immunotherapy with CD3-activated T cells and dendritic cells after surgery: report of a case. Surg Today. 2006;36(6):559-62.
76. Shimizu K, Kotera Y, Aruga A, Takeshita N, Takasaki K, Yamamoto M. Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci. 2012 Mar;19(2):171-8. doi: 10.1007/s00534-011-0437-y.

77. Bylicki O, Barazzutti H, Paleiron N, Margery J, Assié JB, Chouaïd C. First-Line Treatment of Non-Small-Cell Lung Cancer (NSCLC) with Immune Checkpoint Inhibitors. BioDrugs. 2019 Apr;33(2):159-171. doi: 10.1007/s40259-019-00339-4. Review.
78. Di Caro G, Cortese N, Castino GF, Grizzi F, Gavazzi F, Ridolfi C, Capretti G, Mineri R, Todoric J, Zerbi A, Allavena P, Mantovani A, Marchesi F. Dual prognostic significance of tumour-associated macrophages in human pancreatic adenocarcinoma treated or untreated with chemotherapy. Gut. 2016 Oct;65(10):1710-20. doi: 10.1136/gutjnl-2015-309193. Epub 2015 Jul 8.
79. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017 Jul;14(7):399-416. doi: 10.1038/nrclinonc.2016.217.
80. Ohno S, Hiroyuki I, Dhar DJ, Fujii T, Ueda S, Tachibana M, Suzuki N, Inoue M, Soma G, Nagasue N.The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival gastric cancer patients. Anticancer Res 2003; 23:5015-22; PMID:14981961
81. Forssell J, Oberg A, Henriksson ML. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res 2007; 13(5):1472-9; PMID:17332291; https://doi.org/10.1158/1078-0432.CCR-06-2073
82. Ohno S, Ohno Y, Suzuki N, Kamei T, Koike K, Inagawa H, Kohchi C, Soma G, Inoue M. Correlation of histological localization of tumor-associated macrophages with clinicopathological features in endometrial cancer. Anticancer Res 2004; 24:3335-42; PMID:15515429
83. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 2010; 207:2439-53; PMID:20876310; https://doi.org/10.1084/jem.20100587
84. Takeda N, O'Dea EL, Doeden A, Kim JW, Weidemann A, Stockmann C, Asagiri M, Simon MC, Hoffmann A, Johnson RS. Differential activation and antagonistic function of HIF-α isoforms in macrophages are essential for NO homeostasis. Genes Dev 2010; 24:491-501; PMID:20194441; https://doi.org/10.1101/gad.1881410
85. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, Yuan LJ, Hammond R, Gimotty PA, Keith B, Simon MC. Hypoxia-inducible factor 2α regulates macrophage function in mouse models of acute and tumor inflammation. J Clin Invest 2010; 120:2699-714; PMID:20644254; https://doi.org/10.1172/JCI39506
86. Algars A, Irjala H, Vaittinen S, Huhtinen H, Sundström J, Salmi M, Ristamäki R, Jalkanen S. Type and location of tumor-infiltrating macrophages and lymphatic

vessels predict survival of colorectal cancer patients. Int J Cancer. 2012 Aug 15;131(4):864-73. doi: 10.1002/ijc.26457.

87. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, Delaney A, Jones SJ, Iqbal J, Weisenburger DD, Bast MA, Rosenwald A, Muller-Hermelink HK, Rimsza LM, Campo E, Delabie J, Braziel RM, Cook JR, Tubbs RR, Jaffe ES, Lenz G, Connors JM, Staudt LM, Chan WC, Gascoyne RD. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010 Mar 11;362(10):875-85. doi: 10.1056/NEJMoa0905680.
88. Tan KL, Scott DW, Hong F, Kahl BS, Fisher RI, Bartlett NL, Advani RH, Buckstein R, Rimsza LM, Connors JM, Steidl C, Gordon LI, Horning SJ, Gascoyne RD. Tumor-associated macrophages predict inferior outcomes in classic Hodgkin lymphoma: a correlative study from the E2496 Intergroup trial. Blood. 2012 Oct 18;120(16):3280-7. doi: 10.1182/blood-2012-04-421057.
89. Schulz P, Fischer C, Detjen KM, Rieke S, Hilfenhaus G, von Marschall Z, Böhmig M, Koch I, Kehrberger J, Hauff P, Thierauch KH, Alves F, Wiedenmann B, et al. Angiopoietin-2 drives lymphatic metastasis of pancreatic cancer. FASEB J. 2011;25:3325–35.
90. Forget MA, Voorhees JL, Cole SL, Dakhlallah D, Patterson IL, Gross AC, Moldovan L, Mo X, Evans R, Marsh CB, Eubank TD. Macrophage colony-stimulating factor augments Tie2-expressing monocyte differentiation, angiogenic function, and recruitment in a mouse model of breast cancer. PLoS One. 2014;9:e98623.
91. Gabrusiewicz K, Liu D, Cortes-Santiago N, Hossain MB, Conard CA, Aldape KD, Fuller GN, Marini FC, Alonso MM, Idoate MA, Gilbert MR, Fueyo J, Gomez-Manzano C. Anti-vascular endothelial growth factor therapy-induced glioma invasion is associated with accumulation of Tie2-expressing monocytes. Oncotarget. 2014;5:2208–20. doi: 10.18632/oncotarget.1893.
92. Matsubara T, Kanto T, Kuroda S, Yoshio S, Higashitani K, Kakita N, Miyazaki M, Sakakibara M, Hiramatsu N, Kasahara A, Tomimaru Y, Tomokuni A, Nagano H, et al. TIE2-expressing monocytes as a diagnostic marker for hepatocellular carcinoma correlates with angiogenesis. Hepatology. 2013;57:1416–25.
93. Mofarrahi M, McClung JM, Kontos CD, Davis EC, Tappuni B, Moroz N, Pickett AE, Huck L, Harel S, Danialou G, Hussain SN. Angiopoietin-1 enhances skeletal muscle regeneration in mice. Am J Physiol Regul Integr Comp Physiol. 2015;308:R576–89.
94. Satoh N, Yamada Y, Kinugasa Y, Takakura N. Angiopoietin-1 alters tumor growth by stabilizing blood vessels or by promoting angiogenesis. Cancer Sci. 2008;99:2373–9.
95. Atanasov G, Dietel C, Feldbrügge L, Benzing C, Krenzien F, Brandl A, Katou S, Schierle K, Robson SC, Splith K, Wiltberger G, Reutzel-Selke A, Jonas S, Pascher A,

Bahra M, Pratschke J, Schmelzle M. Angiogenic miRNAs, the angiopoietin axis and related TIE2-expressing monocytes affect outcomes in cholangiocarcinoma. Oncotarget. 2018 Jul 6;9(52):29921-29933. doi: 10.18632/oncotarget.25699.

96. Semela D, Dufour JF. Angiogenesis and hepatocellular carcinoma. J Hepatol. 2004;41:864–80.
97. Nowak G, Karrar A, Holmen C, Nava S, Uzunel M, Hultenby K, Sumitran-Holgersson S. Expression of vascular endothelial growth factor receptor-2 or Tie-2 on peripheral blood cells defines functionally competent cell populations capable of reendothelialization. Circulation. 2004;110:3699–707.
98. Goede V, Coutelle O, Shimabukuro-Vornhagen A, Holtick U, Neuneier J, Koslowsky TC, Weihrauch MR, von Bergwelt-Baildon M, Hacker UT. Analysis of Tie2-expressing monocytes (TEM) in patients with colorectal cancer. Cancer Invest. 2012;30:225–30.
99. Puhr HC, Ilhan-Mutlu A. New emerging targets in cancer immunotherapy: the role of LAG3. ESMO Open. 2019 Mar 12;4(2):e000482. doi: 10.1136/esmoopen-2018-000482.
100. Jung K, Heishi T, Khan OF, Kowalski PS, Incio J, Rahbari NN, Chung E, Clark JW, Willett CG, Luster AD, Yun SH, Langer R, Anderson DG, Padera TP, Jain RK, Fukumura D. Ly6Clo monocytes drive immunosuppression and confer resistance to anti-VEGFR2 cancer therapy. J Clin Invest. 2017 Aug 1;127(8):3039-3051. doi: 10.1172/JCI93182.
101. Park JS, Kim IK, Han S, Park I, Kim C, Bae J, Oh SJ, Lee S, Kim JH, Woo DC, He Y, Augustin HG, Kim I, Lee D, Koh GY. Normalization of Tumor Vessels by Tie2 Activation and Ang2 Inhibition Enhances Drug Delivery and Produces a Favorable Tumor Microenvironment. Cancer cell 2016 (30), 953–967; 10.1016/j.ccell.2016.10.018.
102. Mulcahy HE, Toner M, Patchett SE, Daly L, et al. Identifying stage B colorectal cancer patients at high risk of tumor recurrence and death. Dis Colon Rectum 1997; 40:326–331
103. Pollheimer MJ, Kornprat P, Lindtner RA, et al. Tumor necrosis is a new promising prognostic factor in colorectal cancer. Hum Pathol 2010; 41:1749–1757
104. Cona MM, Wang H, Li J, et al. Continuing pursuit for ideal systemic anticancer radiotherapeutics. Invest New Drugs 2012; 30:2050–2065
105. Li JJ, Li J, Sun Z, et al. A dual-targeting anticancer approach: soil and seed principle. Radiology 2011; 260:799–807
106. Valenzuela NM, Reed EF. Antibody-mediated rejection across solid organ transplants: manifestations, mechanisms, and therapies. J Clin Invest. 2017 Jun 30;127(7):2492-2504. doi: 10.1172/JCI90597.

107. Krisl JC, Doan VP. Chemotherapy and Transplantation: The Role of Immunosuppression in Malignancy and a Review of Antineoplastic Agents in Solid Organ Transplant Recipients. Am J Transplant. 2017 Aug;17(8):1974-1991. doi: 10.1111/ajt.14238.

108. Feng S, Bucuvalas J. Tolerance after liver transplantation: Where are we? Liver Transpl. 2017 Dec;23(12):1601-1614. doi: 10.1002/lt.24845.

109. Massart A, Ghisdal L, Abramowicz M, Abramowicz D. Operational tolerance in kidney transplantation and associated biomarkers. Clin Exp Immunol. 2017 Aug;189(2):138-157. doi: 10.1111/cei.12981.

110. Todo S, Yamashita K, Goto R, Zaitsu M, Nagatsu A, Oura T, Watanabe M, Aoyagi T, Suzuki T, Shimamura T, Kamiyama T, Sato N, Sugita J, Hatanaka K, Bashuda H, Habu S, Demetris AJ, Okumura K. A pilot study of operational tolerance with a regulatory T-cell-based cell therapy in living donor liver transplantation. Hepatology. 2016 Aug;64(2):632-43. doi: 10.1002/hep.28459.

## 6. AKNOWLEDGEMENTS

Mein außerordentlicher Dank gilt Herrn Prof. Dr. Johann Pratschke, Direktor der Chirurgischen Klinik der Charité – Universitätsmedizin Berlin, Campus Charité Mitte und Campus Virchow-Klinikum für seine Unterstützung bei der Fertigstellung dieser Arbeit sowie für meine klinische und wissenschaftliche Aus- und Weiterbildung.

Besonders möchte ich mich auch bei Frau Dr. Katrin Schierle aus dem Institut für Pathologie der Universitätsklinik Leipzig für Ihre Supervision und Mithilfe bei den in den wissenschaftlichen Arbeiten durchgeführten histologischen und immunhistologischen Untersuchungen bedanken, ohne die das Forschungsprojekt in dieser Form nicht zustande gekommen wäre. Mein besonderer Dank gilt auch Frau Dr. Corinna Dietel für die Bemühungen und zahlreichen konstruktiven Verbesserungsvorschläge hinsichtlich der aufwendigen Etablierung der immunhistochemischen Methodik. Meine Doktorandinen Charlotte Pöttner und Karoline Dino danke ich zu tiefst für die zahlreichen Stunden fleißiger Arbeit bei der Durchführung der experimentellen Arbeiten des Projektes.

Ein herzlicher Dank gilt ebenfalls den ehemaligen Klinikdirektoren der Klinik und Poliklinik für Viszeral-, Transplantations-, Thorax- und Gefäßchirurgie des Universitätsklinikums Leipzig Prof. Dr. Sven Jonas, Prof. Dr. Uwe Eichfeld und Prof. Dr. Michael Bartels für den ersten Abschnitt meiner klinischen Ausbildung, sowie für die stetige Unterstützung meiner Forschungsaktivitäten.

Meinen Kollegen und Co-Autoren Priv.-Doz. Dr. Christian Benzing und Priv.-Doz. Dr. Felix Krenzien danke ich dafür, dass sie mir während der Fertigstellung der Forschungsprojekten stets mit Rat und Tat zur Seite standen und darüber an der Verfassung der in dieser Schrift zitierten Arbeiten sowie Korrekturlesen der Habilitationsschrift beteiligt waren.

## 7. STATUTORY STATEMENT

*§ 4 Abs. 3 (k) der HabOMed der Charité*

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,

- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,

- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur

Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung

dieser Satzung verpflichte.

Datum Dr. med. Georgi Atanasov