The role of the innate immune system in the development and treatment of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Most patients present with advanced or metastatic HCC at diagnosis and face a dismal prognosis. Tyrosine kinases are the gold standard treatment for this disease but yield limited survival benefits. Immune checkpoint inhibitors that augment adaptive immunity have been tested in HCC. Complex interactions between tumor cells, lymphocytes and the tumor environment determine the efficacy of such immunotherapies. Innate immune mechanisms – known drivers of liver disease progression in pre-HCC conditions such as fibrosis or cirrhosis – may either support or counteract tumor-related immune activation. In this review, we will highlight current concepts of the role of the innate immune system in hepatocarcinogenesis and discuss their relevance for translation into clinics.

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Hepatocellular carcinoma (HCC) is one of the most lethal cancers worldwide. In previous decades both incidence and mortality of liver cancer dramatically increased in industrialized countries, including Europe, North and South America [1]. In up to 90% of cases, HCC arises in cirrhotic or chronically inflamed livers. HCC is considered as the most prominent life-limiting complication of liver cirrhosis [2]. Liver cirrhosis is the common end-stage of chronic liver diseases, such as hepatitis B or C virus infection, alcohol abuse and nonalcoholic fatty liver disease (NAFLD) [3]. Due to the epidemic spread of metabolic diseases, liver cancer is among the fastest growing causes of cancer-related death. A model-based simulation forecasts continued increases in HCC mortality until 2030 underscoring the tremendous relevance of this disease [4].

Despite intensive research activities, most patients with HCC still face a dismal prognosis. Only a minority of patients with early HCC can be scheduled for liver transplantation; patients undergoing alternative curative procedures – such as liver resection or tumor ablation – develop recurrent disease in up to 70% of cases [5]. Patients who are not considered suitable for surgical or interventional strategies, for example, due to advanced tumor stages, can be offered palliative drug therapies including tyrosin kinase inhibitors (TKI) such as sorafenib, lenvatinib, regorafenib or cabozantinib, the VEGFR2-antibody ramucirumab or best supportive care [6-9]. However, these substances yield only marginal survival benefits and are regarded as not cost-effective [10], highlighting the need for novel therapeutic strategies for treatment of HCC.

Chronic inflammation has been shown to promote development of various types of cancer [11,12]. Intensive research has been performed to decipher the immunological mechanisms that are involved in initiation and progression of liver cancer (Table 1). Just recently, immunotherapies have been introduced into clinical treatment algorithms of many cancers such as malignant melanoma, lung cancer and hematologic cancers [13,14], including HCC [15,16]. Intensive research activities have deciphered the immunological mechanisms that are involved in the
Table 1. Role of different innate immune components in hepatocellular carcinoma.

| Immune component                  | Function(s) in HCC                                                                 |
|-----------------------------------|-----------------------------------------------------------------------------------|
| KC                                | Promote immunosuppression and hepatocarcinogenesis                               |
|                                   | Correlate with tumor progression in humans                                       |
| DC                                | Act as messengers between the innate and the adaptive immune systems              |
|                                   | Promote immunotolerance                                                          |
|                                   | Association to initiation and progression of HCC                                 |
|                                   | Indirectly promote proliferation of transformed hepatocytes through inhibitory effects on CD8⁺ T cells |
|                                   | Increased numbers were found in human HCC                                        |
| Myeloid-derived suppressor cells  | Suppress both the arms of immune system: innate and adaptive immunity            |
|                                   | Induced from monocytes/macrophages by differentiation signals from the tumor environment and stellate cells |
|                                   | Support tumor growth                                                             |
|                                   | Promote the expansion of Tregs                                                    |
|                                   | Promote the conversion of CD4⁺ T cells into Tr1 cells                            |
| Neutrophils                       | Promote hepatocarcinogenesis                                                     |
|                                   | Interact with KCs and recruit Tregs as well as macrophages to promote immune tolerance |
|                                   | Promote resistance to sorafenib                                                  |
| NK cells                          | Antifibrotic activity through targeting activated HSCs                           |
|                                   | Diminished function during the development of HCC                                |
|                                   | Promote angiogenesis through MMP9, cytotoxic activity                             |
|                                   | Reduced amounts of NK cells were found in HCC lesions, and the number of CD56⁺ NK cells was correlated with the prognosis in HCC patients |
| NKT cells                         | Directly activate HSCs                                                           |
|                                   | Prevent progression of β-catenin-driven HCC                                      |
|                                   | Involved in the immune surveillance of senescent hepatocytes                      |
|                                   | Increased number in human NASH, cirrhosis and HCC                                |

References are given in the text as well as reviewed in [20,21].

DC: Dendritic cell; HCC: Hepatocellular carcinoma; HSC: Hepatic stellate cell; KC: Kupffer cell; NASH: Nonalcoholic steatosis hepatitis; NK: Natural killer; NKT: Natural killer T cell.

initiation and progression of liver cancer, highlighting the potential of these therapies to treat cancer [17]. In the context of HCC, various studies on the effects of immunotherapies have been conducted with partially conflicting results [18,19], which might be explained by the fact that the efficacy of immunotherapies depends on very complex and only poorly understood interactions between many different immune cells, tumor cells and cells of the tumor environment.

Many of the immune cells infiltrating solid tumors or the tumor microenvironment belong to the innate arm of the immune system [20]. Innovative methodologies such as single-cell RNA sequencing technologies revealed a strikingly heterogeneous composition of immune cells in human liver cirrhosis and HCC, particularly manifold macrophage and dendritic cell (DC) populations, whose distinct functions are only partially understood at present [22,23]. These exciting data fuel the expectation that modulating innate immune cells to improve available immunotherapies or as an alternative immunotherapeutic option might help to overcome limitations of current immunotherapeutic approaches (which largely rely on the adaptive arm of the immune system) in the context of HCC [24]. However, the functionality of innate cells is regulated by several factors, including pro- and anti-inflammatory cytokines as well as fibroblasts, endothelial cells and many other cell types within the tumor or the tumor environment [25]. The complexity of this regulation makes a fast translation of such concepts into clinical routine challenging. In this review, we summarize available data on the role of innate immune responses in hepatocarcinogenesis and discuss its prognostic implications as well as its emerging role as a therapeutic target for HCC.

Current immunotherapeutic approaches for HCC

Numerous immunological approaches have been investigated in the recent past to prevent the development of tumors in cirrhotic livers or to control tumors that have already developed (Table 2). However, the results of immunotherapeutic approaches in HCC have remained, for a long time, largely disappointing. As an example, vaccination strategies have not shown significant benefit, and broad anti-inflammatory treatments did not reduce HCC rates in cirrhotic livers in Phase III trials [26]. In recent years, immune checkpoint blockade has been introduced into therapeutic algorithms of many different cancers [27]. Modulation of checkpoint receptors such as PD-1 or CTLA-4 might reinstate host immune response against malignant tumors, providing tumor control [27]. Both the CheckMate-040 trial and the KEYNOTE-224 trial have reported promising results for the anti-PD-1 antibodies nivolumab and pembrolizumab after failure of TKI therapy in patients with advanced or metastatic...
Table 2. Immunotherapeutic strategies to treat hepatocellular carcinoma.

| Immunotherapy                      | Application details                                           | Mode of action                                                                 |
|------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------|
| Immune checkpoint blockade         | Antibodies (pembrolizumab, ipilimumab, nivolumab, atezolizumab and others) | Inhibition of specific receptors and receptor ligands (PD-1, PD-L1, CTLA-4 and others) to boost antitumor immune responses |
| Vaccines                           | Antigenic peptides/proteins                                  | Tumor-associated antigens are targeted to overcome immune tolerance              |
| Cell therapy                       | Cytokine-induced killer cells, cytotoxic T-lymphocytes, Chimeric antigen receptor T cells | Transfer of tumor-specific T cells from a healthy individual into HCC patients. Patient-derived T cells are modified ex vivo and retransferred into the donor (HCC patient). |

HCC: Hepatocellular carcinoma.

HCC [18,19]. Despite two large Phase III trials failure to confirm results from these earlier trials, the KEYNOTE-240 trial (pembrolizumab does not significantly improve survival in patients with pretreated HCC) and the CheckMate-459 trial (nivolumab does not improve overall survival compared with sorafenib in patients with newly diagnosed unresectable HCC), recent results from the IMBRACE-150 are widely considered as breakthrough for immunotherapy in HCC. Within this study, 501 patients with advanced HCC without prior systemic therapy were randomized to the experimental arm (the anti-PD-1 atezolizumab plus the VEGF inhibitor bevacizumab) or the control arm (sorafenib). The combination therapy significantly improved the overall survival of advanced HCC patients. In the combination arm, the 6-month survival rate was 85% compared with 72% in the sorafenib arm, median survival time was not even achieved in the combination arm so far, while it was 13.2 months in the sorafenib arm (hazard ratio [HR]: 0.55). Similarly, progression-free survival of patients under immunotherapy was significantly better than under sorafenib (6.8 vs 4.3 months; HR: 0.59). With less toxicity, the quality of life of patients on atezolizumab plus bevacizumab was significantly improved and the authors concluded that ‘atezolizumab + bevacizumab should be considered a practice-changing treatment for patients with unresectable HCC who have not received prior systemic therapy,’ highlighting the tremendous potential of immune therapy in patients with HCC.

Nevertheless, even with atezolizumab and bevacizumab, response rates are rather low. At present, the question remains unsolved why checkpoint inhibition does only provide moderate effects in HCC, an inflammation-driven tumor, when compared with other malignancies. However, answers to this question could potentially improve immunotherapy in HCC. Mechanisms potentially mediating resistance against checkpoint inhibition in the setting of HCC might include (but are not limited to) induction of T-cell anergy, inhibition of effector T-cell migration, T-cell inactivation via specific receptor–ligand interactions and barrier functions of the stroma [28–30]. Moreover, macrophages, neutrophils and other immune cells of the innate arm were suggested to be involved in mediating resistance against checkpoint inhibitors [31]. Just recently, a role for tumor-associated neutrophils in mediating resistance against sorafenib was described [32]. In line, Cheng et al. suggested that activated PD-L1+ neutrophils might exert a protumor effect by suppressing T-cell immunity in a PD1/PD-L1-dependent manner within the tumor microenvironment, highlighting the role of the innate arm of the immune systems in mediating response to PD(L)-1 directed therapies in HCC [33]. Besides neutrophils, recent results indicated an important role of natural killer (NK) cells, which express immunological checkpoint molecules such as PD-1 and CTLA-4 to a significant extent. PD-1/PD-L1 blockade has been shown to augment NK cell-mediated tumor lysis in multiple myeloma. Moreover, it was suggested that targeting PD-1/PD-L1 might also activate NK cells [34]. Besides PD-1 and CTLA-4, TIM-3 and LAG-3 represent potential NK cell immune checkpoints (summarized in [35]). Both have emerged as targets for cancer immunotherapy, due to their capacity of negatively regulating T-cell activation and synergizing with PD-1 to exhaust T cells [36]. At present, several ongoing clinical trials are exploring the therapeutic efficacy of LAG-3 and PD-1 combined treatment with various advanced cancers [35]. Thus, cells of the innate arm of the immune systems might not only affect efficacy of current immunotherapies but are also represent targets for novel immunotherapeutic approaches in cancer.

Role of innate immune cells for hepatocarcinogenesis & immunotherapy

In recent years, tumor-infiltrating immune cells have been intensively analyzed and characterized (Table 1). For numerous solid tumors, including primary liver cancer, associations between certain immune cell populations and response to therapy as well as on prognosis have been proposed [17,37–39]. Although the precise significance of the tumor immune microenvironment is still not fully understood, a high density of myeloid cells is often associated
with a poor prognosis and a high density of infiltrating T-effector cells is often associated with a good prognosis [40,41]. Thus, understanding the immune microenvironment may predict, guide and improve immunotherapy [42].

During the progression of liver diseases, inflammation is considered a driving factor and a prerequisite for liver cancer [43]. Some of these ‘tumor-promoting’ aspects of inflammation in chronic liver diseases include hepatocyte cell death followed by aberrant regeneration, fibrosis or angiogenesis [44]. Technological advances such as single-cell RNA sequencing provide a more in-depth understanding of cellular heterogeneity in the inflamed environment of a fibrotic or cirrhotic liver [45–48]. However, malignant tumors also create an intrinsic inflammatory response, favoring antitumor responses in some of the cases [12,49].

Monocytes & macrophages
The hepatic immune response provoked by HCC has been examined in different mouse models and retraced in blood and tissue samples from patients with HCC. For instance, the lack of T- and/or B-cells increases chemically induced liver cancer, supporting that adaptive immune responses limit hepatocarcinogenesis [50]. On the other hand, a prolonged activation of both Kupffer cells (KCs) and inflammatory monocytes represents a characteristic (pathological) feature observed in the context of chronic liver inflammation [51], potentially leading to HCC. In this setting, chemokine CCL2/chemokine receptor CCR2-dependent signaling mechanisms were suggested to be involved in the process of hepatocarcinogenesis. CCL2 is expressed at higher levels in patients with HCC, and elevated CCL2 expression levels were indicative for an impaired prognosis [29]. In murine models of liver cancer, CCR2+ myeloid cells exert a context-dependent function: while they have a protective role via clearance of senescent (premalignant) hepatocytes and CCR2 ablation was associated with increased tumor burden, they were demonstrated to have a tumor-promoting role through NK cell inhibition once HCC established [52]. Interestingly, treatment with a CCR2 antagonist inhibited HCC growth and metastasis, ultimately enhancing survival in a model of senescence-associated HCC [52], while in a model of nonalcoholic steatohepatitis (NASH)-dependent HCC, CCR2 depletion had no clear effect on HCC tumorigenesis [53], suggesting that the effect of CCR2 in hepatocarcinogenesis depend on disease etiology. Functionally, blocking of the CCL2/CCR2 axis was associated to an inhibition of the recruitment of inflammatory monocytes, infiltration and repolarization (‘M2’) of tumor-associated macrophages and an activation of an antitumorous CD8+ T-cell response [54]. These CCR2-dependent macrophages also promote pathogenic angiogenesis for tumor vascularization in fibrotic livers [55]. The group of Yao et al. extracted a novel CCR2 inhibitor, named 747, from Abeis georgei. Administration of 747 to HCC-bearing mice was associated with an increase of intratumoral CD8+ T cells leading to an activation of antitumoral immune responses and to tumor control in orthotopic and subcutaneous HCC. Moreover, 747 demonstrated additive effects when used in combination with sorafenib, indicating that the immunomodulators might represent a therapeutic option to improve current treatment strategies in the context of HCC [56]. From a clinical perspective, inhibiting CCL2/CCR2 axis is a realistic therapeutic option in the near future, because the dual chemokine receptor CCR2/CCR5 inhibitor cenicriviroc is under development for patients with NASH and fibrosis [57]. This drug effectively reduces the recruitment of inflammatory monocytes to injured livers, which ameliorates hepatic fibrosis [51,58]. The potential benefits for HCC development are, however, currently unknown.

Kupffer cells
A role for KCs, the resident macrophages in the liver, in hepatocarcinogenesis was described in the context of chemical carcinogen-induced liver cancer, where they promote carcinogenesis in a complex and yet not fully understood interaction with hepatocytes [59]. Dying hepatocytes might release danger signals (‘DAMP’) triggering activation of KCs via Toll-like receptors. Moreover, increasing evidence is available for an activation of KCs via upregulation of HIF-1α, occurring in the context of liver hypoxia [60]. Another activating pathway for KCs that appears to be highly relevant during hepatocarcinogenesis is the hyaluronan-CD44-dependent binding of platelets. The therapeutic inhibition of platelet cargo, platelet adhesion and platelet activation resulted in reduced KC activation, improved NASH phenotype and reduced carcinogenesis in mouse models of HCC [61]. KC, but also monocyte-derived macrophages, releases cytokines like TNF that triggers liver cancer by inducing mitochondrial dysfunction and reactive oxygen species accumulation [62].

Deciphering the exact roles of KCs and monocyte-derived macrophages (as a prerequisite for specific therapeutic targeting) is further hampered by the heterogeneity of these myeloid cells regarding origin/ontogeny, activation pathways and functions in health and disease [63]. This has been demonstrated in the highly prevalent conditions of NAFLD and NASH, in which HCC might occur even in the absence of liver cirrhosis. During progression of
NAFLD upon western diet feeding in mice, single-cell RNA sequencing analyses revealed a unique inflammatory activation profile among different myeloid cell populations in liver and simultaneously in bone marrow, which has implications for injury recognition, metabolism and immune activation [46]. Following the detailed description of macrophages in the liver, many pathways were identified that contribute to the proinflammatory polarization of macrophages during NASH, including changes in the gut microbiota, an altered metabolism that diminish glucocorticoid signaling or epigenetic changes [64,65]. Within macrophages, the formation of inflammasome complexes promotes inflammatory activation, and inhibition of the NLRP3 molecule protected from NASH in rodent models [66]. Intriguingly, activation of inflammasomes provide a link to the development of liver cancer in NASH via production of IL-6, which has a mitogenic effect on hepatocytes and promotes HCC [67]. In addition, activated hepatic macrophages promote NASH and the development of HCC through the production of reactive oxygen species [68–70].

Myeloid-derived suppressor cells

The functionality of immune cells in the microenvironment of malignant tumors represents a highly regulated process. In the context of HCC, monocyte-derived macrophages were identified to contribute to the recognition and clearance of senescent (premalignant) hepatocytes, which prevents tumor development [52]. In the environment of an established HCC, monocyte-derived cells can acquire a phenotype of myeloid-derived suppressor cells (MDSCs), which support tumor growth [71]. Tumor-induced MDSCs demonstrate positivity for the myeloid surface marker CD11b and the granulocyte/monocyte marker Gr1 in mice [72]. Various studies described the hepatic enrichment of CD11b+Gr1+ immune cells in experimental liver cancer and of similar cells in human HCC [71]. MDSCs promote the expansion of Tregs and promote the conversion of CD4+ T cells into T regulatory cells. In HCC, Tregs mediate immune tolerance and promote immune escape [73]. Besides Tregs, DCs might promote immunotolerance and thereby lead to initiation and progression of HCC [74,75]. Targeting MDSCs, for example, by blocking their T-cell-suppressing activity, could therefore be attractive to enhance the efficacy of immunotherapy. This has been exemplarily demonstrated in mouse models of liver cancer, in which suppressing MDSC enhanced the therapeutic efficacy of ‘cytokine-induced killer cells’ that were administered to tumor-bearing mice [76].

More recently, several studies addressed interactions between hepatic stellate cells (HSCs) and monocytes/macrophages for understanding mechanisms driving the development of MDSCs. Using coculture systems, mouse models and human samples for validation, HSCs were found to induce MDSC differentiation in the liver tumor environment [77,78]. Activated HSCs induced p38 MAPK signaling in macrophages, which promoted their reprogramming toward MDSC development that suppressed (antitumoral) T-cell activity. In turn, interfering with the HSC–MDSC interaction by the p38 MAPK inhibitor reduced HCC growth in preclinical models [78]. These data principally demonstrate that interfering with the innate immune environment of HCC could potentiate immunotherapeutic approaches.

Neutrophils

Neutrophils represent the most abundant leukocytes in peripheral blood. Moreover, neutrophils are among the most common tumor-infiltrating immune cells. Recent data have suggested that neutrophils play an important role in the regulation of tumor initiation and progression [79,80]. In line, elevated numbers of neutrophils are associated to an impaired prognosis of patients with solid tumors in general [80] and in patients with HCC in particular [81]. Mechanistically, it was demonstrated in different non-HCC tumor entities that neutrophils promote tumor cell proliferation [82], tumor vascularization, migration and invasion [83–85]. Moreover, neutrophils might suppress antitumor immunity [86] by producing a plethora of prooncogenic ligands [87]. Recently, neutrophils were also suggested to promote hepatocarcinogenesis (e.g. [32]). In HCC, tumor-associated neutrophils closely interact with KCs and recruit Tregs as well as macrophages [32], thereby leading to immune tolerance. More specifically, immunohistochemical analysis revealed that CCL2+ and CCL17+ cells, coexpressing CD66b (thereby marking them as neutrophilic cells), were enriched within the HCC microenvironment and correlated with tumor size, microvascular invasion, tumor encapsulation, tumor differentiation and stage. Strikingly, higher levels of these cells indicated an unfavorable prognosis in HCC patients [32]. In mice, tumor-associated neutrophils promoted resistance to sorafenib, while in turn combining sorafenib with a neutrophil-inhibiting agent provided superior tumor control than sorafenib alone [32]. Thus, inhibition of tumor-associated neutrophils might represent a novel therapeutic concept using cells from the innate arm of the immune system. In line to these data, Cheng et al. recently demonstrated that the phenotype and function of neutrophils in HCC is influenced by cancer-associated...
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fibroblasts (CAF) [33]; neutrophils were activated by conditioned medium from CAF with increased expression of CD66b, PD-L1, IL-8, TNF-α and CCL2, and with decreased expression of CD62L. CAF-primed neutrophils impaired T-cell function through the PD-1/PD-L1 signaling pathway [33], highlighting a mechanism by which cells from the innate arm of the immune system might affect response to checkpoint inhibition in HCC.

NKT cells

NKT cells represent a subpopulation of lymphocytes that are activated very early in an immune response but lack immunological memory. As such, most authors attribute NKT cells to the innate immune system ('innate lymphocytes') rather than to the adaptive immune system. When activated, NKT cells produce Th1 or Th2 cytokines [30]. Our group has recently shown that iNKT cells are recruited in a CXCR6-dependent manner to the liver already during the very early phase of immune response in different models of liver injury [88]. CXCR6⁺ NKT cells in turn activate local macrophages, thereby further enhancing inflammation [89]. NKT cells are also involved in antitumor immunity. NKT cells prevent development of tumor metastasis in manifold cancers. Moreover, iNKT cells from patients with malignant diseases secrete less IFN-γ compared with tiNKT cells derived from healthy controls [90]. The specific role of NKT cells in hepatocarcinogenesis is less well understood. Just recently, our group demonstrated that CXCR6-deficient mice had a significantly higher tumor burden than wild-type mice in a control [90]. The specific role of NKT cells in hepatocarcinogenesis is less well understood. NK cell activation represents a highly regulated process that is driven by many different components of the tumor environment. As such, not only abiotic components (hypoxia, metabolites, acidosis, cytokines and growth factors) influence hepatic NK cells but also biotic players including stromal cells, regulatory immune cells and ‘normal’ neighboring cells contribute to NK cell activation. Moreover, a whole plethora of chemokines might trigger or inhibit NK cell homing to the malignant lesion, since they are equipped with several chemokine receptors [101]. In addition, NK cells are not only influenced by the microenvironment but themselves shape the tumor microenvironment (nicely summarized in [102]). Therefore, additional research is needed before a therapeutic use of NK cells to prevent hepatocarcinogenesis can be considered.

NK cells

In HCC, the main cytotoxic interactions seem to be mediated by CD8⁺ T cells and CD57⁺ NK cells [20,98]. NKT cells, DCs and KCs activate NK cells, while Tregs and HSCs prevent the activation of NK cells [99]. Reduced amounts of NK cells were found in HCC lesions [100]. However, the role of NK cells in HCC is only partially understood. NK cell activation represents a highly regulated process that is driven by many different components of the tumor environment. As such, not only abiotic components (hypoxia, metabolites, acidosis, cytokines and growth factors) influence hepatic NK cells but also biotic players including stromal cells, regulatory immune cells and ‘normal’ neighboring cells contribute to NK cell activation. Moreover, a whole plethora of chemokines might trigger or inhibit NK cell homing to the malignant lesion, since they are equipped with several chemokine receptors [101]. In addition, NK cells are not only influenced by the microenvironment but themselves shape the tumor microenvironment (nicely summarized in [102]). Therefore, additional research is needed before a therapeutic use of NK cells to prevent hepatocarcinogenesis can be considered.

Other cells in the tumor microenvironment

Finally, nonparenchymal cells also modulate immune responses in HCC. For instance, HSCs inhibit T-cell activation by inducing MDSC [103] and by recruiting Tregs [104]. Liver sinusoidal endothelial cells activate Tregs via the TGF-β, inducing an immunosuppressive liver environment [105]. The role of so-called tumor-associated fibroblasts is not so well defined in HCC. Tumor-associated fibroblasts can inhibit antitumor response by impairing the function of NK cells and by facilitating MDSC generation in the tumor microenvironment [106].
Conclusion
The adaptive and innate immune system represents highly specialized but closely interconnected systems that maintain the integrity of the liver and protect it against external threats. In chronic liver diseases, innate immune mechanisms critically contribute to persistent inflammation, fibrosis and cirrhosis, thereby providing the seeding ground for hepatocarcinogenesis. In malignant diseases, innate and adaptive immunity are part of a highly differentiated tumor microenvironment, which can have both pro- and anti-carcinogenic effects and thus contribute decisively to the effectiveness of antitumor therapy. In this context, influencing the immune system and thereby shaping the tumor microenvironment toward the anticancerogenic side might help to control tumor growth and to improve patients' prognosis.

Future perspective
Current immunotherapies mainly rely on the adaptive arm of the immune system. A better understanding of the innate arm of the immune system might help to develop novel concepts with improved efficacy. Targeting innate immune mechanisms has the potential to improve immunosurveillance in inflamed liver, reduce HCC development in liver cirrhosis and boost conventional immune checkpoint inhibitor strategies in established HCC. Such novel concepts might help to 'find the holy grail' in immuno-oncology, namely, to turn immunologically 'cold' tumors 'hot.' At present, combinations of anti-PD-L1/-anti-PD-1-directed therapies are tested in numerous clinical trials. For instance, inhibiting the VEGFR pathway was shown not only to reprogram the tumor microenvironment but also to increase efficient priming and activation of T-cell responses enhancing response to anti-PD-L1/-anti-PD-1 antibodies. In line, the IMBRAVE-150 trial that analyzed atezolizumab plus bevacizumab in patients with advanced HCC revealed a significantly higher efficacy of this combination compared with sorafenib. Moreover, different trials are investigating the role of MEK inhibition in the context of immunoncology, since MEK inhibition potentially increases recruitment of cytotoxic T cells into the tumor core, potentially reversing T-cell exhaustion. Finally, measures to increase contact of immune cells toward tumor antigens (radiation therapy, ablation, embolization) are analyzed in combination with anti-PD-L1/-anti-PD-1 antibodies within large Phase III trials.

Summary points
- Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is still associated to a dismal prognosis.
- HCC represents an inflammation-driven disease and immune-oncological approaches are likely to improve patients' prognosis. However, recent Phase III trials have failed to demonstrate a survival benefit of anti-PD-1/-PD-L1-directed therapies.
- In recent years, tumor-infiltrating immune cells have been extensively analyzed and characterized. Associations between distinct immune cell populations and response to therapy as well as on prognosis have been proposed in patients with HCC.
- The liver comprises a large population of innate immune cells in the body such as resident macrophages (Kupffer cells), natural killer cells or natural killer T cells, highlighting the important roles of these cells in the liver and in hepatocarcinogenesis.
- Influencing the innate immune system and thereby shaping the tumor microenvironment toward anticancerogenic actions might help to control tumor growth and to improve patients' prognosis.
- Combination therapies are tested in numerous clinical trials and most likely represent the future of immuno-oncology in HCC.

Author contributions
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