

## REVIEW

# New insights into the pathogenesis and treatment of non-viral hepatocellular carcinoma: a balancing act between immunosuppression and immunosurveillance

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## Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide. HCC initiates as a consequence of chronic liver damage and inflammation caused by hepatitis B and C virus infections, excessive alcohol consumption, or non-alcoholic fatty liver disease (NAFLD). Until recently, no effective treatments for advanced HCC were available and the 5-year survival rate had remained below 8% for many years. New insights into the mechanisms that drive the development of NAFLD-related HCC indicate that loss of T-cell-mediated immunosurveillance plays a cardinal role in tumor growth and malignant progression, in addition to previously identified inflammation-driven compensatory proliferation. Recently completed groundbreaking clinical studies have shown that treatments that restore antitumor immunity represent a highly effective therapeutic option for approximately 20% of advanced HCC patients. Understanding the causes of inflammation-driven immunosuppression and immune system dysfunction in the 80% of patients who fail to reignite antitumor immunity despite treatment with checkpoint inhibitors should lead to further and even more dramatic improvements in HCC immunotherapy.

## Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and second leading cause of cancer-related deaths worldwide.<sup>1</sup> Despite a general decline in cancer-related deaths, HCC incidence in the USA and its associated mortality continue to grow at an alarming

rate, with a tripling of HCC-related mortality in the past 30 years.<sup>2</sup> Historically, the main causes of HCC were hepatitis B and C virus (HBV, HCV) infections, but the incidence of virus-related HCC is predicted to decline within the next generation because of development of effective and economical HBV vaccines and the recent

Received: 23 January 2018; Revised: 21 March 2018; Accepted: 7 May 2018

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introduction of highly effective anti-HCV drugs.<sup>1,3</sup> By contrast, the incidence of non-viral hepatitis continues to rise and it is already a leading cause of cryptogenic cirrhosis and liver transplantation in the USA and Europe.<sup>4,5</sup> Major causes of non-viral hepatitis include the obesity epidemic, which has greatly increased the incidence of non-alcoholic fatty liver disease (NAFLD), and rampant alcohol abuse, which results in alcoholic liver disease (ALD).<sup>6</sup> Surgical resection and liver transplantation represent effective treatment options for early, localized HCC, but unfortunately the majority of HCC cases are diagnosed at a rather advanced stage, and are frequently associated with loss of liver function. Compromised liver function means that advanced HCC cannot be treated with high doses of chemotherapy or ionizing radiation and the only evidence-based targeted therapeutic approved for first-line HCC treatment is the pan-kinase inhibitor sorafenib, which extends life by less than 3 months without an impact on 5-year survival rates, which have persisted at <8% for stage 4 patients.<sup>7</sup> Furthermore, sorafenib is a highly hepatotoxic drug that is not suitable for all patients.

Until recently, the only meaningful improvements in HCC treatment have been: 1) the establishment of strict criteria for performance of liver resection and liver transplantation,<sup>8,9</sup> and 2) patient classification and stratification, which help identify those who will benefit most from first-line sorafenib treatment.<sup>10,11</sup> This lack of progress in HCC treatment is most likely because of the absence of reliable biomarkers that allow for effective screening of high-risk patients and early disease detection. This poor state of affairs is likely to change. Recent clinical studies have demonstrated that single-agent treatment with immune checkpoint inhibitors such as nivolumab and pembrolizumab, antibodies that block engagement of the inhibitory receptor PD-1, which is expressed by exhausted CD8<sup>+</sup> T cells, led to a significant decrease in tumor burden in ~20% of advanced HCC patients.<sup>12,13</sup> These findings are entirely consistent with the results of a recent preclinical study which showed that loss of T-cell-mediated immunosurveillance from inflammation-driven immunosuppression plays a cardinal role in the development of NAFLD-associated HCC.<sup>14</sup> This incredible and rare convergence between mechanistic preclinical research and empirical clinical investigation strongly suggests that immunotherapies aimed at restoring HCC immunosurveillance will revolutionize the treatment of this highly aggressive malignancy and may also lead to its prevention in high-risk individuals.

### Preclinical models for understanding HCC etiology and pathogenesis

Currently, the majority of HCC cases in the USA and Europe are caused by HCV hepatitis, non-alcoholic steatohepatitis (NASH), or alcoholic steatohepatitis (ASH).<sup>1</sup>

However, in the not-too-distant future, the prevalent HCC etiologies in Western society are predicted to be associated with non-viral hepatitides (especially NASH and ASH), that are driven by the ever-expanding obesity epidemic<sup>4</sup> and excessive alcohol consumption. HBV- or HCV-induced HCC has been difficult to study in mice, because these viruses do not replicate in non-primate mammals. Early attempts to study HBV- and HCV-induced HCC in mice were based on transgenic expression of whole virus genomes or parts of them from liver-specific promoters.<sup>15–18</sup> Although HBV and HCV transgenic mice develop HCC after a considerable latency, it is questionable whether the tumorigenic mechanisms operating in these mice are related to those that function in HBV- or HCV-infected humans. While HBV is a DNA tumor virus that is thought to induce HCC through insertional mutagenesis,<sup>19</sup> HCV is a non-integrating RNA virus that does not code for any oncogene.<sup>20</sup> It has been speculated that HCV replication within hepatocytes causes endoplasmic reticulum (ER) stress that leads to induction of hepatic steatosis, chronic liver damage, and inflammation, outcomes that have also been detected in HCV transgenic mice.<sup>21,22</sup>

Curiously, ER stress was also suggested to be associated with the most severe manifestation of NAFLD—NASH<sup>23–26</sup>—suggesting that HCV infection and NASH may lead to HCC development through a common pathogenic mechanism that involves ER stress. To determine whether hepatocyte ER stress can contribute to HCC development, we chose to use *MUP-uPA* transgenic mice, which express urokinase plasminogen activator (uPA) from the liver-specific major urinary protein (MUP) promoter.<sup>26</sup> By exceeding the folding and secretory capacity of hepatocytes in the newborn liver, uPA expression results in ER stress and transient liver damage that subsides after 6 weeks of age because of transgene extinction.<sup>26,27</sup> By placing 6-week-old *MUP-uPA* mice on a high-fat diet (HFD), we were able to reignite the ER stress response and cause activation of sterol response element binding proteins (SREBP), thereby enhancing the accumulation of liver triglycerides (TG) and cholesterol within hepatocytes.<sup>28,29</sup> Whereas TG accumulation leads to simple hepatic steatosis, the buildup of free, unesterified cholesterol acts in conjunction with TNF, which is produced by activated liver macrophages,<sup>29</sup> to amplify the extent of liver inflammation and damage. Thus, both free cholesterol and TNF serve as critical factors in controlling the transition from hepatic steatosis or early NAFLD to NASH in both mice and humans.<sup>30–33</sup> Indeed, within several months of HFD initiation, *MUP-uPA* mice develop extensive liver inflammation manifested by massive leukocytic infiltration, chronic liver damage, and hepatocyte death, which result in compensatory proliferation, a “chicken-wire” pattern of fibrosis, and accumulation of Mallory-Denk Bodies (MDB), which are inclusion bodies composed of p62-containing protein aggregates. Of note, MDB serve

as a typical sign of chronic liver diseases associated with increased risk of HCC development,<sup>29,34</sup> but as discussed below, their main constituent, p62, is also a cause of hepatic tumorigenesis. All of these responses are characteristic of human NASH<sup>24</sup> and are entirely dependent on TNF signaling via the type I TNF receptor (TNFR1) that is expressed on the surface of hepatocytes. Genetic ablation of TNFR1 or TNF titration using Enbrel (etanercept) completely prevents NASH development in HFD-fed MUP-uPA mice.<sup>29</sup> Anecdotal clinical reports indicate that Enbrel or Remicade (infliximab) treatment lead to improvement of NASH symptoms in both animal models and human patients,<sup>35–38</sup> although this should be contrasted with the failure of such drugs in alcoholic hepatitis.<sup>18</sup> It should be noted, however, that ASH patients are much more likely to develop severe infections than NASH patients, thereby precluding the use of anti-TNF drugs, which otherwise had shown promising results.<sup>39</sup> Importantly, within 9 months of HFD initiation, at least 85% of HFD-fed MUP-uPA male mice (HCC is much more common in males than in females<sup>40</sup>) show numerous, poorly differentiated HCC nodules with about 50% of them having the appearance of steatotic HCC.<sup>29</sup>

Consistent with early results published by the Pikarsky group,<sup>41</sup> TNFR1 signaling via I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ) also plays an important role in NASH to HCC progression by stimulating the proliferation of HCC progenitor cells (HcPC).<sup>29</sup> Another important player in NASH to HCC progression is p62/SQSTM1. Hepatocyte-specific ablation of the *Sqstm1* gene largely attenuates HCC development in HFD-fed MUP-uPA mice.<sup>34</sup> In addition to its propensity for forming protein aggregates, MDB, and hyaline granules,<sup>42</sup> p62 is an important signaling protein<sup>43</sup> whose tumorigenic activity is exerted via activation of transcription factor NRF2. A point mutation in the KIR motif of p62, which prevents its binding to KEAP1, the negative regulator of NRF2,<sup>44</sup> also blocks the ability of overexpressed p62 to induce HCC development.<sup>34</sup> p62-mediated NRF2 activation also plays an important role in the development of pancreatic cancer, stimulating the malignant progression of preneoplastic PanIN1 lesions.<sup>45</sup> Gain-of-function mutations in the NRF2-encoding *NFE2L2* gene and loss-of-function mutations in the KEAP1 gene, both of which lead to constitutive NRF2 activation, were detected in up to 12% of human HCC specimens.<sup>46,47</sup>

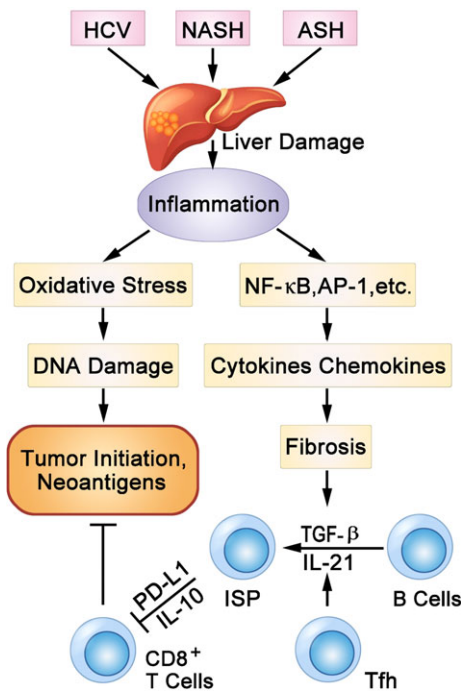
In addition to sharing identical pathological features and common oncogenic signaling pathways, HCCs that have appeared in MUP-uPA mice are essentially identical to human HCC in their mutational signature, which exhibited a marked enrichment for C to T transitions.<sup>14</sup> The mutational load of mouse NASH-driven HCC is also quite similar to that of human HCC, averaging 50–100 coding region point mutations per tumor. Many of these mutations affect oncogenic drivers that were first detected in human HCC.<sup>14,46,47</sup>

## The oncogenic role of inflammation-driven immunosuppression

Another important feature of both NASH and ASH that is closely associated with liver fibrosis is the presence of high amounts of circulating immunoglobulin A (IgA).<sup>48,49</sup> In both NASH and ASH, the serum concentration of IgA is directly proportional to the extent of liver fibrosis.<sup>48</sup> HFD-fed MUP-uPA mice and other mouse models of liver fibrosis or NASH exhibit a positive correlation between circulating IgA and liver fibrosis.<sup>14</sup> Circulating IgA in NASH-afflicted mice is produced by liver-infiltrating plasma cells that have undergone IgM to IgA class switch recombination (CSR) in response to TGF- $\beta$  and other cytokines, such as IL-21 and IL-33, whose expression is elevated in response to chronic liver inflammation.<sup>14</sup> Using freshly collected liver biopsies, we confirmed the presence of liver-infiltrating IgA-expressing plasma cells in human NASH and have shown that these cells, as well as their progenitors, IgA<sup>+</sup> plasmablasts, also express the inhibitory ligand PD-L1 and the immunosuppressive cytokine IL-10.<sup>14</sup> Using the MUP-uPA + HFD model we have developed, we demonstrated that these cells, collectively referred to as immunosuppressive plasmacytes (ISPs), are the principal source of PD-L1 in NASH-driven HCC and are directly responsible for inducing the exhaustion of HCC-directed CD8<sup>+</sup> T cells.<sup>14</sup> Ablation of the IgA locus or inhibition of ISP generation through interference with TGF- $\beta$  signaling resulted in attenuation of HCC development and concomitant reinvigoration of HCC-directed cytotoxic T cells (CTLs). Depletion of CTLs using a CD8 neutralizing antibody restored NASH-induced HCC development in IgA-deficient mice.<sup>14</sup> A dramatically reduced tumor load was observed after treatment of HCC-bearing MUP-uPA mice with a PD-L1 blocking antibody, but the few tumors that did develop in ISP-deficient mice were completely refractory to PD-L1 blockade.<sup>14</sup> These results indicate that IgA<sup>+</sup> ISP, which accumulate in response to chronic liver damage, inflammation, and fibrosis, are the most critical source of PD-L1 in HCC, a tumor whose development is strongly dependent on the suppression of CTL-mediated immunosurveillance. In support of these conclusions, HCC development is highly accelerated in MUP-uPA/*Cd8a*<sup>-/-</sup> mice, which lack CTL.<sup>14</sup> HCC development is also accelerated in MUP-uPA/*Rag1*<sup>-/-</sup> mice, whose reconstitution with T cells in the absence of B cells inhibits tumor development. We conclude that HCC-directed liver-infiltrating CD8<sup>+</sup> T cells are potent inhibitors of HCC emergence because of their ability to recognize and kill HCC progenitors. These findings are summarized in Fig. 1.

## The PD-1 checkpoint and the therapeutic effect of its inhibition

PD-L1 is the main ligand for the inhibitory receptor PD-1 (programmed death-1). PD-1 is expressed by CD8<sup>+</sup>



**Figure 1.** Key molecular elements that dictate the balance between tumor immunity and inflammation-driven immunosuppression. Tfh, follicular helper T cells; ISP, immunosuppressive plasmocytes.

cytotoxic T cells as well as CD4<sup>+</sup> T follicular helper cells (Tfh), whereas PD-L1 can be expressed by many different cell types, including macrophages, B cells, and epithelial cells.<sup>50,51</sup> The engagement of PD-1 on the surface of CD8<sup>+</sup> T cells inhibits their proliferation and all of their effector functions, thereby resulting in a dysfunctional state that is commonly referred to as exhaustion.<sup>52,53</sup> Importantly, this inhibitory response is needed to prevent the rampant activation of antiviral CTLs, and its absence can result in severe collateral damage and even mortality in response to viral infections.<sup>54</sup> In fact, myeloid and epithelial cells begin to express PD-L1 in response to interferon  $\gamma$  (IFN- $\gamma$ ) that is produced by activated CTLs, thereby constituting a negative feedback loop. Engagement of PD-1 on the surface of Tfh cells also inhibits cell proliferation but it also leads to induction of IL-21 and other molecules through which Tfh stimulate the maturation of plasma cells, including IgA-expressing plasma cells<sup>55,56</sup> (Fig. 1).

In addition to the control of antiviral immunity and Tfh function, it was found that PD-1:PD-L1 interactions play a cardinal role in antitumor immunity. Many types of cancer express PD-L1 on their surface and thereby lead to the exhaustion of tumor-directed CTL.<sup>57,58</sup> Consistent with these observations, it was found that treatment of tumor-bearing mice with antibodies against either PD-1 or PD-L1 that block the interaction between the two molecules triggers tumor regression and concomitant reactivation/reinvigoration of tumor-directed CD8<sup>+</sup> T cells.<sup>59,60</sup> These impressive preclinical results and the success of blocking antibodies directed against another T-cell checkpoint regulator CTLA4<sup>61</sup>

quickly led to clinical trials of the first fully human PD-1 blocking antibody, nivolumab, which resulted in an objective response rate (ORR) of 17% in non-small cell lung cancer.<sup>62,63</sup> Since then, nivolumab, pembrolizumab, and the PD-L1 antagonistic antibody atezolizumab, have received approvals for the treatment of melanoma, lung cancer, bladder cancer, kidney cancer, head and neck cancer, and Hodgkin lymphoma.<sup>50,64</sup> It was initially postulated that only cancers with high mutational load can be treated with checkpoint inhibitor drugs, including PD-L1 inhibitors,<sup>63,65,66</sup> but this short-lived dogma has not prevented oncologists from testing these drugs in cancers with lower mutational loads, such as renal cell carcinoma<sup>67</sup> and HCC.<sup>12</sup> Surprisingly, the response rates to all PD-1:PD-L1 inhibitors were found to cluster around 15–25% and do not seem proportional to the total number of mutations a particular tumor harbors.<sup>50</sup> Clearly, mutational load is not the only factor that affects the response to checkpoint inhibitors. Another factor suggested to affect the response to PD-1:PD-L1 interaction inhibitors is the level of PD-L1 expression on tumor cells.<sup>50,62</sup> Although PD-L1 expression is needed for activation of the PD-1 checkpoint and to make the involved tumor responsive to PD-1:PD-L1 inhibitors, the degree of PD-L1 expression by cancer cells themselves was not found to directly correlate with response rates. Furthermore, the source of PD-L1 is highly variable and many cancers show PD-L1 expression by components of the tumor microenvironment, rather than the malignant cells themselves.<sup>68,69</sup> Correlating PD-L1 expression with response rates to PD-1:PD-L1 antagonists has not been highly reliable because of the mediocre quality of the reagents and methodology used to assess PD-L1 expression. Given that in non-viral HCC the most critical source of PD-L1 are the IgA<sup>+</sup> ISP,<sup>14</sup> it is not surprising that no correlation was found between the response to nivolumab and PD-L1 expression on HCC cells.<sup>12</sup> We postulate that the presence of elevated serum IgA and liver-infiltrating IgA<sup>+</sup> ISP can be a much more accurate parameter for predicting responsiveness to PD-1/PD-L1 targeting agents in human HCC. Elevated circulating IgA has also been detected in HBV- and HCV-infected patients<sup>70–73</sup> and is much easier to measure than to quantitate PD-L1 expression in tissue/tumor sections.

## PD-1:PD-L1 blockade: a revolution in HCC treatment

As mentioned above, PD-1/PD-L1 blocking therapies were thought to be irrelevant for the treatment of HCC because the typical HCC mutational load is lower than the cutoff value postulated to be needed for anti-PD-1/PD-L1 responsiveness.<sup>63,66</sup> Furthermore, hepatitis is a common side effect of checkpoint inhibitor therapy<sup>74,75</sup> and it was therefore assumed that advanced HCC patients would not be able to tolerate such a complication. These considerations, however, did not prevent El-Khoueiry and colleagues from conducting the CheckMate



040 trial, the first clinical study that clearly demonstrated the effectiveness of nivolumab monotherapy in advanced HCC.<sup>12</sup> The phase 1/2 CheckMate 040 clinical study enrolled 262 patients with histologically confirmed advanced HCC, including patients with non-viral HCC, HCV-infected, and HBV-infected patients. Of these groups, patients with non-viral hepatitis or HCV hepatitis were observed to exhibit an ORR of 20–25%, whereas HBV-infected patients exhibited an ORR of 14%.<sup>12</sup> Although the study was insufficiently powered to correlate response rates with etiology, the results suggest that HBV-infected patients are actually less responsive to PD-1 blockade therapy than non-virus-infected patients or HCV-infected patients. This is somewhat counterintuitive because HBV infection should result in expression of viral antigens that are potent T-cell activators. Of note, the CheckMate 040 study found no correlation whatsoever between membrane expression of PD-L1 by HCC cells and the response to PD-1 blockade,<sup>12</sup> further demonstrating the weakness of the hypothesis according to which the response to anti-PD-1 drugs is determined by PD-L1 expression on the surface of cancer cells. The clinical findings reported by El-Khoueiry *et al.* are fully consistent with the finding of our preclinical study of NASH-driven HCC and its control by the interaction between IgA<sup>+</sup> ISP and CD8<sup>+</sup> CTLs.<sup>14</sup> The mouse studies demonstrated that PD-L1 mediated exhaustion of HCC-targeting CD8<sup>+</sup> T cells plays a critical role in HCC development, therefore suggesting that the PD-1 checkpoint is key to HCC development. Despite low PD-L1 expression by HCC cells, the use of nivolumab resulted in disease control in 67% of patients with non-viral HCC or HCV-related HCC and 55% of patients with HBV-related HCC. These striking results should be compared with response rates of 2–3% in HCC patients treated with first-line sorafenib.<sup>76,77</sup> The median duration of the response to nivolumab was as high as 17 months in the dose-escalation phase of the trial,<sup>12</sup> far exceeding the 3-month extension in survival offered by sorafenib. Even the early fear of nivolumab-induced hepatitis has not panned out. Only two patients out of 202 who completed the trial experienced acute hepatitis and the overall rate of adverse events was not any higher than in any other population of similarly treated cancer patients. Thus, there is no question that anti-PD-1/PD-L1 therapies will become the game-changers that will revolutionize HCC treatment. Shortly after completion of the CheckMate 040 trial, nivolumab was approved for the treatment of advanced HCC when used following prior treatment with sorafenib. Hopefully, future studies will show nivolumab, pembrolizumab, and similar drugs are suitable for first-line HCC treatment without prior administration of sorafenib, which has no effect on the rate or duration of the therapeutic response.<sup>12</sup> Similar and more extensive responses were seen in HCC-bearing *MUP-uPA* mice that were treated with a PD-L1 blocking antibody.<sup>14</sup> We initiated anti-PD-L1 treatment after 7 months of HFD feeding, a time point at which the majority of *MUP-uPA* mice exhibit visibly detectable liver tumors. After 8 weeks of 3 injections per week

with two different PD-L1 antibodies, only one of which is a functional blocking antibody, we observed that PD-L1 blockade led to 60% reduction in tumor load relative to control.<sup>14</sup> The therapeutic effect of PD-L1 blockade was most noticeable on larger tumors, most of which had disappeared. In addition to demonstrating the utility of PD-L1 blockade in a mouse model that is amenable to detailed mechanistic analysis, this experiment has taught us several very important lessons. First, tumor-bearing mice that lack PD-L1-expressing ISP did not show any response to PD-L1 blockade, indicating that in NASH-driven HCC, at least in mice, the critical functional source of PD-L1 are the ISP. Although HCC tumor cells express small amounts of PD-L1, that particular PD-L1 is not functionally important. These results are entirely consistent with those of the CheckMate 040 trial. Second, tumors in *MUP-uPA/Cd8a<sup>-/-</sup>* mice also did not respond to anti-PD-L1 treatment, indicating that the targets for PD-1/PD-L1 blockade are the exhausted CD8<sup>+</sup> T cells. Indeed, anti-PD-L1 treatment of wildtype *MUP-uPA* mice decreased the liver content of exhausted CD8<sup>+</sup> T cells and increased the number of proliferating and degranulating effector CD8<sup>+</sup> T cells that express TNF, IFN- $\gamma$ , granzyme B, and perforin.<sup>14</sup> These results indicate that CD8<sup>+</sup> T cells not only prevent HCC initiation through immunosurveillance, but also that they are responsible for the rejection of established tumors in response to treatment with PD-1: PD-L1 blocking antibodies. In other words, tumors that do not contain exhausted CD8<sup>+</sup> T cells are unlikely to be responsive to PD-1/PD-L1 blockade.

The preclinical mouse studies may have also provided us with a precious clue that could explain why no more than 25% of HCC patients mount a response to nivolumab treatment. We observed that the few HCC-bearing mice that did not show a response to PD-L1 blockade contained tumors that were surrounded by an envelope of activated hepatic stellate cells (HSC), the kind of cells that are responsible for extracellular matrix deposition during liver fibrosis.<sup>14</sup> In addition to the envelope of activated HSC, these tumors contained very few infiltrating T cells and most of the reinvigorated CD8<sup>+</sup> effector T cells stayed outside of the tumor. In this respect, these treatment refractory tumors resembled pancreatic cancer, which contains an extensive stroma of activated pancreatic stellate cells and is devoid of invading CD8<sup>+</sup> T cells.<sup>78</sup> Of note, we found that similar to HCC, both mouse and human pancreatic adenocarcinomas contain PD-L1-expressing IgA<sup>+</sup> plasmacytes. The significance of these findings remains to be determined, but they suggest that somehow HSC or pancreatic stellate cells interfere with the activation of CTL or their ability to penetrate the tumor and recognize their target.

## Conclusions and future directions

The rare confluence of preclinical and clinical studies described above strongly establishes the relevance of

antitumor immunity to the development and treatment of HCC, one of the most common and difficult-to-treat malignancies. It has been known for many years that HCC development is dependent on chronic liver damage and inflammation, but until now it was assumed that the main pro-tumorigenic effect of liver damage is compensatory proliferation, a biological response that stimulates the division of transformed hepatocytes.<sup>79,80</sup> The new studies reviewed above indicate that another important effect of chronic liver damage and the ensuing inflammatory response is the suppression of CTL-mediated immunosurveillance. As long as it is left unperturbed, immunosurveillance provides very strong protection against the growth of nascent HCC lesions, which emerge from mutated pericentral hepatocytes.<sup>81</sup> Given the key pro-tumorigenic effect of inflammation-driven immunosuppression, a process that depends on accumulation of PD-L1 expressing ISP, it is no wonder that drugs that block the binding of PD-L1 to PD-1 and restore CTL-mediated antitumor immunity are so remarkably effective in the treatment of advanced HCC.

Despite the incredible clinical advance in HCC treatment represented by PD-1:PD-L1 interaction inhibitors, the average objective response to this class of drugs is approximately 20%.<sup>12</sup> Undoubtedly, this rate needs to be increased, but how can this be accomplished? I suggest that, first and foremost, we need to understand the factors that render the remaining 80% of HCC patients non-responsive to PD-1/PD-L1 blockade. Given the dependence of the response to PD-L1 blockade on the presence of IgA<sup>+</sup> ISP,<sup>14</sup> it is plausible that some of the non-responsive patients may have low amounts of liver- and HCC-resident ISP. As circulating IgA is easy to measure and known to directly correlate with liver IgA,<sup>14</sup> one simple study that needs to be carried out in the very near future is a correlative study between serum IgA and the response to PD-1/PD-L1 blockade. It is also important to determine how many of the non-responsive patients exhibit excessive accumulation of activated HSC around their tumors and defective tumoral invasion of reinvigorated CD8<sup>+</sup> T cells. If the findings made in mice are validated in a significant portion of the anti-PD-1 non-responsive patient population, it will become important to test the effect of clinically approved drugs that are capable of inhibiting HSC activation. At this point two classes of such drugs come to mind: 1) phosphodiesterase 5 (PDE5) inhibitors, and 2) vitamin D analogs. PDE5 inhibitors, such as tadalafil (Cialis) and sildenafil (Viagra), were previously observed to inhibit HSC activation and prevent the accumulation of prostate and lung myofibroblasts, which are highly similar in their properties to activated HSC.<sup>82,83</sup> In fact, tadalafil has been approved for the treatment of benign prostatic hyperplasia and pulmonary hypertension, two diseases that depend on myofibroblast activation.<sup>84,85</sup> Likewise, the vitamin D analog calcipotriol was found to attenuate HSC activation and interfere with their ability to express numerous chemokines and other molecules,<sup>86,87</sup> including CXCL13, a B-cell chemoattractant, that may account

for the immunosuppressive activity of HSC and other types of myofibroblasts. Importantly, drugs in both groups are safe and free of side effects that could reduce the effectiveness of PD-1/PD-L1 targeting drugs. Another important line of future research pertains to the involvement of other checkpoint inhibitors, such as antibodies to TIM-3, LAG-3, and CTLA4, in controlling the immune response to HCC. So far such studies have not been reported, but it is plausible that blockade of additional inhibitory receptors may result in more sustained reinvigoration of tumor-directed CD8<sup>+</sup> T cells than has been seen with PD-1 blockade alone.<sup>88</sup>

## Acknowledgements

Research in M.K.'s laboratory is supported by the NIH/NCI (R01CA211794), the NIEHS Superfund Research Program (P42ES010337), and the Tower Cancer Research Foundation (Senior Investigator Grant). M.K. holds the Ben and Wanda Hildyard Chair for Mitochondrial and Metabolic Diseases.

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