Table 2. *Cont.*

Cancer	Immune Suppressive Cells		Treatments, Future Treatments or Other Uses	References
	Tregs	MDSCs		
non-small-cell lung cancer	○		prognostic biomarker	Hasegawa *et al.* [53]
				Yukawa *et al.* [54]
		○	prognostic biomarker	Liu *et al.* [43]
Lewis lung carcinoma (mouse model)	○		anti-miR214	Yin *et al.* [55]
Pancreatic ductal adenocarcinoma	○		prognostic biomarker	Luardi *et al.* [57]
Renal Cell Carcionam	○	○	High-dose IL2 followed by Sorafenib	Monk *et al.* [47]
		○	prognostic biomarker	Mirza *et al.* [58]
		○	prognostic biomarker	Kusmartsev *et al.* [59]
Solitary Fibrous Tumor		○	Sunitinib malate	Tazzari *et al.* [60]
Thyroid cancer	○		Inhibition of FOXP3	Chu *et al.* [61]
Glioblastoma	○		prognostic marker	Soyour E *et al.* [62]
Colon carcinoma (mouse model)	○	○	c-kit antibody	Pan *et al.* [63]

Recently, nucleoside analogues have emerged as an important treatment option for chronic hepatitis B patients [64]. However, discontinuation of nucleoside analogues could frequently induce the reactivation of chronic hepatitis [65,66]. Therefore, pegylated interferon (Peg-IFN) could be a significant treatment to control replication of HBV by inducing an immune response. The efficacy of Peg-IFN treatment has not yet become optimal [67]. Immune suppressive cells including Tregs and MDSCs might contribute to the difficulty of inducing an effective immune response for HBV persistent infection and HCC. This review focuses on the Tregs and MDSCs that could be potential targets for the immune therapy of chronic hepatitis B patients and HCC.

2. Tregs Could Affect HBV Persistent Infection

In addition to hepatitis c virus infection (HCV), it has been reported that the HBV-specific immune response could be suppressed by $CD4^+CD25^+$ Tregs in patients with HBV infection [68]. This report indicated that not only Tregs from CH-B patients, but also those from patients with resolved HBV infection could suppress HBV specific $CD8^+$ T cell. However, it has been reported that the frequency of Tregs in CH-B patients was significantly higher than those in healthy controls and those with resolved HBV infection [69]. Therefore, the frequency of Tregs might contribute to the disease status of HBV infection [23,24,68–76] (Table 3). Tregs have been identified by using CD4, CD25, CD45RO and CTLA-4 antibodies. Another group reported that the frequency of $CD39^+$Tregs correlates with the progression of HBV infection [77]. Therefore, we should consider this minor subset of Tregs in chronic hepatitis B patients [77]. Previously, we reported that HBcAg-specific IL10 secreting $CD4^+CD25^+$ Tregs might contribute to the suppression of HBV-specific IFN-gamma secreting $CD4^+$ T cells [23].

Moreover, the depletion of Tregs could recover the function of IFN-gamma secretion of $CD4^+$ T cells in an *ex vivo* study [23]. Another group reported a similar phenomenon and the enhancement of HBV-specific T cell proliferation after the depletion of Tregs [69]. Previously, several groups including ours reported that the reduction of HBV could recover the frequency of HBV-specific T cells and the function of T cells [15,78]. Tregs might contribute to suppressing the HBV-specific T cells. Therefore, treatment with a nucleos(t)ide analogue might affect the Tregs. Previously, Stoop *et al.* [71] described that adefovir-induced viral load reduction caused a decrease in circulating Tregs together with a partial recovery of the immune response. They described that the frequency of Tregs among $CD4^+$ T cells was decreased at three and six months after adefovir treatment. Moreover, they determined that the frequency of HBcAg-specific IFN-gamma secreting cells was increased during adefovir treatment. We also reported that the entecavir-induced viral load reduction caused a decrease in circulating Tregs [24]. Moreover, we analyzed the mechanism of Tregs enhancement of functions in chronic hepatitis B patients [24]. Heat shock protein 60 produced from HBV-replicative hepatocytes might enhance the IL 10-secreting function of Tregs via toll like receptor 2 (TLR2). The inhibition of TLR2 signaling could inhibit the excessive function of Tregs. Another group reported that over-expression of TLR2/4 on monocytes could modulate the activities of Tregs in chronic hepatitis B patients [79]. This report described that the agonists of TLR2 and 4 activated-Tregs showed enhanced suppression function in chronic hepatitis B patients. Another group reported that exogenous tumor necrosis factor alpha partially abrogated the Tregs-mediated suppression [80]. The interaction between programmed death (PD)-1 and its ligand, PD-L1, is important for the induction of exhausted T cells. In chronic hepatitis B patients, the antiviral intrahepatic T cell response could be restored by blocking the PD-1 pathway [81]. Tregs express both PD-L1 and PD-1 [82]. That PD-L1/PD-1 signaling might suppress the HBV-specific immune response has been reported by many groups [81,83–86]. The inhibition of PD-1 and cytotoxic lymphocyte antigen-4 (CTLA-4) could slightly enhance the cellular proliferation and significantly increased the IFN-gamma production of PBMCs co-cultured with Tregs [84]. Concerning the $CD4^+$ development pathway, both induced Tregs and Th17 cells require TGF-β. In addition to TGF-β, IL-2 promotes development of Tregs and inhibits Th17 cells, whereas IL6, IL21 and IL23 promote the development of Th17 cells and inhibit that of Tregs. Therefore, the balance between Tregs and Th17 cells during hepatitis B virus infection was analyzed [87–90]. A group reported that acute or chronic HBV-related liver failure patients have a dramatically higher $IL17^+/FOXP3^+$ ratio than that in chronic liver failure patients [87]. Another report described that a lower Treg/Th17 ratio induced greater liver fibrosis progression [88]. Although these findings are unsurprising, the balance between Tregs and Th17 might be usefully analyzed for the disease status and treatment response. A group described that the frequency of Tregs increased in non-responders but not in responders during pegylated-interferon [91]. The frequency and/or function of Tregs could affect the natural course of chronic hepatitis B patients and treatment response [92]. Most of the groups analyzed the peripheral blood to detect Tregs (Table 3). Some groups analyzed liver-infiltrating lymphocytes in addition to peripheral blood to detect Tregs. Xu *et al.* [70] indicated that the frequency of liver-infiltrating Tregs increased in CH-B patients and chronic severe hepatitis B patients, as seen in peripheral blood. Yang *et al.* [72] reported that Foxp3+ cells were present in significantly higher numbers in liver tissue sections from chronic active hepatitis B, as seen in peripheral blood. However, it is difficult to carry out the sequential analysis of liver-infiltrating lymphocytes since liver biopsy has a risk of bleeding.

Table 3. Association between HBV infection and Tregs.

Species or Model	Disease Status	Immune Subset	Frequency, Functions or Findings	References
Human	AHB Recovered	Isolated $CD4^+CD25^+$	Suppression of $CD8^+$ cells	Franzese *et al.* [68]
	CHB		Frequecny (AHB = CHB = Healthy)	
	Healhty subjects			
Human	AHB Recovered	Isolated $CD4^+CD25^+$	Suppression of $CD4^+$ cells	Stoop *et al.* [69]
	CH-B		Frequency(Chronic > recovered: Chronic > healthy donors)	
	Healthy subjects			
Human	AH-B	Isolated $CD4^+CD25^+$; $FOXP3^+$ gated liver infiltrating lymphocytes	Suppression of $CD4^+$ cells	Xu *et al.* [70]
	CH-B		Frequency(CH-B severe > CHB, CHB severe > AH-B, CH-B severe > healthy donors)	
	Healthy subjects			
Human	CH-B	Isolated $CD4^+$ $CD25^+$	Suppression of $CD4^+$ cells	Kondo *et al.* [23]
	Healthy subjects		Chronic = healthy donors	
Human	Treated CH-B	$CD4^+$ $CD25^+$ $CTLA4^+$ $CD45RO^+$ ($FOXP3^+$)	Frequency (Treated CHB < CHB)	Stoop *et al.* [71]
	CH-B			
	Healthy subjects			
Human	Recovered AH-B	Isolated $CD4^+CD25^+$; $FOXP3^+$ liver infiltrating lymphocytes	Suppression of $CD4^+$ cells and $CD8^+$ cells	Yang *et al.* [72]
	CH-B		Frequency(Chronic asymptomatic > chronic active > resolved = healthy controls)	
	Healthy subjects			
Human	CH-B	$CD4^+CD25^+IL7R^-$	sHSP60 enhances Tregs activity via TLR2 signaling	Kondo *et al.* [24]
	Treated CH-B patients		Frequency (Treated CHB < CHB)	
	Healthy subjects			
Woodchuck hepatits	Woodchuck hepatitis	$CD4^+FOXP3^+$	Frequency (WHV > Control)	Otano *et al.* [73]
HBV model			Interleukin-12 Increases Hepatic Tolerogenicity	

Table 3. *Cont.*

Species or Model	Disease Status	Immune Subset	Frequency, Functions or Findings	References
Mouse	AdHBV	$CD4^+FOXP3^+$	Down-regulating the antiviral activity of effector T cells by limiting cytokine production and cytotoxicity	Stross *et al.* [74]
Human	HBV-HCC	$CD4^+CD25^+FOXP3$	TGF-b-miR-34a-CCL22 Signaling-Induced Treg Cell Recruitment	Yang *et al.* [75]
Human	CH-B	$CD4^+CD25^+$	Frequency (CH-B = Acute on choronic HBV = Healthy)	Dong *et al.* [76]
	Acute on choric HBV			
	Healthy			

3. Tregs and HBV-Related HCC

It has been reported that the frequency of Tregs is increased in HCC patients [93,94]. Yang *et al.* [93] analyzed the frequency of Tregs and $CD8^+$ T cell in peripheral blood and liver tissue. The results indicated a significant increase in both the proportion and absolute numbers of $CD4^+CD25^+$ T-cells in the peri-tumor region [94]. Another group indicated the higher frequency of Tregs in the peripheral blood from HCC patients in comparison to those from HCV patients and healthy subjects. The mechanisms of increased Tregs in HCC were analyzed. Huh7 culture supernatants appear to promote $CD4^+CD25^+$ T-cell proliferation and inhibit $CD4^+CD25^-$ T-cell proliferation [95]. Moreover, the frequency of Tregs could be a significant biomarker of survival in HCC patients. The frequency of circulating $CD4^+CD25^+FoxP3^+$ Tregs was increased significantly and correlated with the disease progression in HBV-related HCC patients [22,96,97]. An abundant accumulation of Tregs concurrent with a significantly reduced infiltration of $CD8^+$ T cells was found in tumor regions compared with nontumor regions [96]. Another group reported that the frequency of the $CD45RO^+$ subset in $CD4^+CD25^{high}$ Tregs was associated with progression of HCCs [98]. Moreover, the frequency of the other phenotype of Tregs ($CD4^+CD25^-CD69^+$) was also increased in HCC patients [99]. The induction of Tregs could be affected by not only hepatitis B virus infection but also HCC. When PBMCs were co-cultured with human hepatoma cell lines stably transfected with HBV (HepG2.2.15), the $CD4^+CD25^+$ Tregs population increased and upregulated Tregs-related genes [100]. Sorafenib is a multikinase inhibitor that could suppress cell proliferation and angiogenesis. Sorafenib could reduce the frequency of hepatic infiltrating Tregs by suppressing TGF-β signaling [101]. Suppressing Tregs might be one of the significant targets for the induction of immunity for HCC.

4. MDSCs for HBV Persistent Infection and HBV-Related HCC

An emerging cell population of interest, MDSCs, could contribute to immune suppression. In a mouse model, it has been reported that liver-derived MDSCs from HBV transgenic mice could suppress the proliferative capacities of allogenic T cells and HBsAg-specific lymphocytes [34].

Recently, it has been reported that γδT cells could drive MDSCs-mediated $CD8^+$ T cell exhaustion in HBV persistent infection [33]. In addition to the suppressive function of MDSCs, MDSCs could affect the induction and function of Tregs [19]. It has been reported that MDSCs could induce Tregs in HCC patients [19]. Moreover, another group reported that higher frequencies of $GARP^+CTLA\text{-}4^+Foxp3^+$ Tregs and MDSCs in HCC patients are associated with impaired T-cell functionality [20]. Compared to Tregs, few reports have described the relationship between MDSCs and HCC. However, many groups including ours are focusing on MDSCs for the induction-mechanism of Tregs in HBV persistent infection and HBV-related HCC.

5. Concluding Remarks

Many groups including ours have reported that Tregs and MDSCs suppress the immune reaction for HBV and HCC. The excessive activation of immune suppressive cells could contribute to the persistent infection of HBV and the progression of HCC. Therefore, detailed mechanisms of the induction of Tregs and MDSCs should be investigated to control HBV persistent infection and HBV-related HCC. Moreover, the ability to specifically suppress Tregs and MDSCs, and understanding the appropriate time point to do so, might improve the treatment of HBV-related diseases.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Tiollais, P.; Pourcel, C.; Dejean, A. The hepatitis B virus. *Nature* **1985**, *317*, 489–495.
2. Lai, C.L.; Ratziu, V.; Yuen, M.F.; Poynard, T. Viral hepatitis B. *Lancet* **2003**, *362*, 2089–2094.
3. Liaw, Y.F.; Brunetto, M.R.; Hadziyannis, S. The natural history of chronic HBV infection and geographical differences. *Antivir. Ther.* **2010**, *15*, 25–33.
4. Maeshiro, T.; Arakaki, S.; Watanabe, T.; Aoyama, H.; Shiroma, J.; Yamashiro, T.; Hirata, T.; Hokama, A.; Kinjo, F.; Nakayoshi, T.; *et al.* Different natural courses of chronic hepatitis B with genotypes B and C after the fourth decade of life. *World J. Gastroenterol.* **2007**, *13*, 4560–4565.
5. Kondo, Y.; Ueno, Y.; Shimosegawa, T. Toll-like receptors signaling contributes to immunopathogenesis of HBV infection. *Gastroenterol. Res. Pract.* **2011**, *2011*, doi:10.1155/2011/810939.
6. Sato, S.; Li, K.; Kameyama, T.; Hayashi, T.; Ishida, Y.; Murakami, S.; Watanabe, T.; Iijima, S.; Sakurai, Y.; Watashi, K.; *et al.* The RNA Sensor RIG-I Dually Functions as an Innate Sensor and Direct Antiviral Factor for Hepatitis B Virus. *Immunity* **2015**, *42*, 123–132.
7. Kakimi, K.; Guidotti, L.G.; Koezuka, Y.; Chisari, F.V. Natural killer T cell activation inhibits hepatitis B virus replication *in vivo*. *J. Exp. Med.* **2000**, *192*, 921–930.
8. Bonorino, P.; Ramzan, M.; Camous, X.; Dufeu-Duchesne, T.; Thelu, M.A.; Sturm, N.; Dariz, A.; Guillermet, C.; Pernollet, M.; Zarski, J.P.; *et al.* Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C. *J. Hepatol.* **2009**, *51*, 458–467.

9. Kondo, Y.; Ninomiya, M.; Kakazu, E.; Kimura, O.; Shimosegawa, T. Hepatitis B surface antigen could contribute to the immunopathogenesis of hepatitis B virus infection. *ISRN Gastroenterol.* **2013**, *2013*, doi:10.1155/2013/935295.
10. Baron, J.L.; Gardiner, L.; Nishimura, S.; Shinkai, K.; Locksley, R.; Ganem, D. Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection. *Immunity* **2002**, *16*, 583–594.
11. Boltjes, A.; Groothuismink, Z.M.; van Oord, G.W.; Janssen, H.L.; Woltman, A.M.; Boonstra, A. Monocytes from chronic HBV patients react *in vitro* to HBsAg and TLR by producing cytokines irrespective of stage of disease. *PLoS One* **2014**, *9*, e97006.
12. Huang, Y.W.; Hsu, C.K.; Lin, S.C.; Wei, S.C.; Hu, J.T.; Chang, H.Y.; Liang, C.W.; Chen, D.S.; Chen, P.J.; Hsu, P.N.; *et al.* Reduced Toll-like receptor 9 expression on peripheral $CD14^+$ monocytes of chronic hepatitis B patients and its restoration by effective therapy. *Antivir. Ther.* **2014**, *19*, 637–643.
13. Chisari, F.V.; Ferrari, C. Hepatitis B virus immunopathogenesis. *Annu. Rev. Immunol.* **1995**, *13*, 29–60.
14. Reignat, S.; Webster, G.J.; Brown, D.; Ogg, G.S.; King, A.; Seneviratne, S.L.; Dusheiko, G.; Williams, R.; Maini, M.K.; Bertoletti, A. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J. Exp. Med.* **2002**, *195*, 1089–1101.
15. Kondo, Y.; Asabe, S.; Kobayashi, K.; Shiina, M.; Niitsuma, H.; Ueno, Y.; Kobayashi, T.; Shimosegawa, T. Recovery of functional cytotoxic T lymphocytes during lamivudine therapy by acquiring multi-specificity. *J. Med. Virol.* **2004**, *74*, 425–433.
16. Martinet, J.; Dufeu-Duchesne, T.; Bruder Costa, J.; Larrat, S.; Marlu, A.; Leroy, V.; Plumas, J.; Aspord, C. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. *Gastroenterology* **2012**, *143*, 1586–1596.
17. Shi, B.; Ren, G.; Hu, Y.; Wang, S.; Zhang, Z.; Yuan, Z. HBsAg inhibits IFN-alpha production in plasmacytoid dendritic cells through TNF-alpha and IL-10 induction in monocytes. *PLoS One* **2012**, *7*, e44900.
18. Ma, Y.J.; He, M.; Han, J.A.; Yang, L.; Ji, X.Y. A clinical study of HBsAg-activated dendritic cells and cytokine-induced killer cells during the treatment for chronic hepatitis B. *Scand. J. Immunol.* **2013**, *78*, 387–393.
19. Hoechst, B.; Ormandy, L.A.; Ballmaier, M.; Lehner, F.; Kruger, C.; Manns, M.P.; Greten, T.F.; Korangy, F. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces $CD4^+CD25^+Foxp3^+$ T cells. *Gastroenterology* **2008**, *135*, 234–243.
20. Kalathil, S.; Lugade, A.A.; Miller, A.; Iyer, R.; Thanavala, Y. Higher frequencies of $GARP^+CTLA\text{-}4^+Foxp3^+$ T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. *Cancer Res.* **2013**, *73*, 2435–2444.

21. Han, Y.; Chen, Z.; Yang, Y.; Jiang, Z.; Gu, Y.; Liu, Y.; Lin, C.; Pan, Z.; Yu, Y.; Jiang, M.; Zhou, W.; Cao, X. Human $CD14^+CTLA\text{-}4^+$ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. *Hepatology* **2014**, *59*, 567–579.
22. Wang, F.; Jing, X.; Li, G.; Wang, T.; Yang, B.; Zhu, Z.; Gao, Y.; Zhang, Q.; Yang, Y.; Wang, Y.; Wang, P.; Du, Z. $Foxp3^+$ regulatory T cells are associated with the natural history of chronic hepatitis B and poor prognosis of hepatocellular carcinoma. *Liver Int.* **2012**, *32*, 644–655.
23. Kondo, Y.; Kobayashi, K.; Ueno, Y.; Shiina, M.; Niitsuma, H.; Kanno, N.; Kobayashi, T.; Shimosegawa, T. Mechanism of T cell hyporesponsiveness to HBcAg is associated with regulatory T cells in chronic hepatitis B. *World J. Gastroenterol.* **2006**, *12*, 4310–4317.
24. Kondo, Y.; Ueno, Y.; Kobayashi, K.; Kakazu, E.; Shiina, M.; Inoue, J.; Tamai, K.; Wakui, Y.; Tanaka, Y.; Ninomiya, M.; *et al.* Hepatitis B virus replication could enhance regulatory T cell activity by producing soluble heat shock protein 60 from hepatocytes. *J. Infect. Dis.* **2010**, *202*, 202–213.
25. Suri-Payer, E.; Amar, A.Z.; Thornton, A.M.; Shevach, E.M. $CD4^+CD25^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J. Immunol.* **1998**, *160*, 1212–1218.
26. Shen, S.L.; Liang, L.J.; Peng, B.G.; He, Q.; Kuang, M.; Lai, J.M. $Foxp3^+$ regulatory T cells and the formation of portal vein tumour thrombus in patients with hepatocellular carcinoma. *Can. J. Surg.* **2011**, *54*, 89–94.
27. Chen, W.; Jin, W.; Hardegen, N.; Lei, K.J.; Li, L.; Marinos, N.; McGrady, G.; Wahl, S.M. Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J. Exp. Med.* **2003**, *198*, 1875–1886.
28. Hori, S.; Nomura, T.; Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **2003**, *299*, 1057–1061.
29. Natarajan, S.; Thomson, A.W. Tolerogenic dendritic cells and myeloid-derived suppressor cells: Potential for regulation and therapy of liver auto- and alloimmunity. *Immunobiology* **2010**, *215*, 698–703.
30. Lu, L.R.; Liu, J.; Xu, Z.; Zhang, G.L.; Li, D.C.; Lin, C.S. Expression and clinical significance of myeloid derived suppressor cells in chronic hepatitis B patients. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 4367–4372.
31. Ostrand-Rosenberg, S.; Sinha, P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *J. Immunol.* **2009**, *182*, 4499–4506.
32. Peranzoni, E.; Zilio, S.; Marigo, I.; Dolcetti, L.; Zanovello, P.; Mandruzzato, S.; Bronte, V. Myeloid-derived suppressor cell heterogeneity and subset definition. *Curr. Opin. Immunol.* **2010**, *22*, 238–244.
33. Kong, X.; Sun, R.; Chen, Y.; Wei, H.; Tian, Z. Gammadelta T cells drive myeloid-derived suppressor cell-mediated $CD8^+$ T cell exhaustion in hepatitis B virus-induced immunotolerance. *J. Immunol.* **2014**, *193*, 1645–1653.
34. Chen, S.; Akbar, S.M.; Abe, M.; Hiasa, Y.; Onji, M. Immunosuppressive functions of hepatic myeloid-derived suppressor cells of normal mice and in a murine model of chronic hepatitis B virus. *Clin. Exp. Immunol.* **2011**, *166*, 134–142.

35. Anthony, D.D.; Umbleja, T.; Aberg, J.A.; Kang, M.; Medvik, K.; Lederman, M.M.; Peters, M.G.; Koziel, M.J.; Overton, E.T. Lower peripheral blood $CD14^+$ monocyte frequency and higher $CD34^+$ progenitor cell frequency are associated with HBV vaccine induced response in HIV infected individuals. *Vaccine* **2011**, *29*, 3558–3563.
36. Hoechst, B.; Voigtlaender, T.; Ormandy, L.; Gamrekelashvili, J.; Zhao, F.; Wedemeyer, H.; Lehner, F.; Manns, M.P.; Greten, T.F.; Korangy, F. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology* **2009**, *50*, 799–807.
37. Kapanadze, T.; Gamrekelashvili, J.; Ma, C.; Chan, C.; Zhao, F.; Hewitt, S.; Zender, L.; Kapoor, V.; Felsher, D.W.; Manns, M.P.; *et al.* Regulation of accumulation and function of myeloid derived suppressor cells in different murine models of hepatocellular carcinoma. *J. Hepatol.* **2013**, *59*, 1007–1013.
38. Schmidt, K.; Zilio, S.; Schmollinger, J.C.; Bronte, V.; Blankenstein, T.; Willimsky, G. Differently immunogenic cancers in mice induce immature myeloid cells that suppress CTL *in vitro* but not *in vivo* following transfer. *Blood* **2013**, *121*, 1740–1748.
39. Tacke, R.S.; Lee, H.C.; Goh, C.; Courtney, J.; Polyak, S.J.; Rosen, H.R.; Hahn, Y.S. Myeloid suppressor cells induced by hepatitis C virus suppress T-cell responses through the production of reactive oxygen species. *Hepatology* **2012**, *55*, 343–353.
40. Zeng, Q.L.; Yang, B.; Sun, H.Q.; Feng, G.H.; Jin, L.; Zou, Z.S.; Zhang, Z.; Zhang, J.Y.; Wang, F.S. Myeloid-derived suppressor cells are associated with viral persistence and downregulation of TCR zeta chain expression on $CD8^+$ T cells in chronic hepatitis C patients. *Mol. Cells* **2014**, *37*, 66–73.
41. Watanabe, S.; Deguchi, K.; Zheng, R.; Tamai, H.; Wang, L.X.; Cohen, P.A.; Shu, S. Tumor-induced $CD11b^+Gr\text{-}1^+$ myeloid cells suppress T cell sensitization in tumor-draining lymph nodes. *J. Immunol.* **2008**, *181*, 3291–3300.
42. Hanson, E.M.; Clements, V.K.; Sinha, P.; Ilkovitch, D.; Ostrand-Rosenberg, S. Myeloid-derived suppressor cells down-regulate L-selectin expression on $CD4^+$ and $CD8^+$ T cells. *J. Immunol.* **2009**, *183*, 937–944.
43. Liu, C.Y.; Wang, Y.M.; Wang, C.L.; Feng, P.H.; Ko, H.W.; Liu, Y.H.; Wu, Y.C.; Chu, Y.; Chung, F.T.; Kuo, C.H.; *et al.* Population alterations of L-arginase- and inducible nitric oxide synthase-expressed $CD11b^+/CD14^-/CD15^+/CD33^+$ myeloid-derived suppressor cells and $CD8^+$ T lymphocytes in patients with advanced-stage non-small cell lung cancer. *J. Cancer Res. Clin. Oncol.* **2010**, *136*, 35–45.
44. Ahearne, M.J.; Bhuller, K.; Hew, R.; Ibrahim, H.; Naresh, K.; Wagner, S.D. Expression of PD-1 (CD279) and FoxP3 in diffuse large B-cell lymphoma. *Virchows Arch.* **2014**, *465*, 351–358.
45. Pinheiro, D.; Chang, Y.M.; Bryant, H.; Szladovits, B.; Dalessandri, T.; Davison, L.J.; Yallop, E.; Mills, E.; Leo, C.; Lara, A.; *et al.* Dissecting the regulatory microenvironment of a large animal model of non-Hodgkin lymphoma: Evidence of a negative prognostic impact of $FOXP3^+$ T cells in canine B cell lymphoma. *PLoS One* **2014**, *9*, e105027.

46. Braga, W.M.; da Silva, B.R.; de Carvalho, A.C.; Maekawa, Y.H.; Bortoluzzo, A.B.; Rizzatti, E.G.; Atanackovic, D.; Colleoni, G.W. *FOXP3* and *CTLA4* overexpression in multiple myeloma bone marrow as a sign of accumulation of $CD4^+$ T regulatory cells. *Cancer Immunol. Immunother.* **2014**, *63*, 1189–1197.
47. Monk, P.; Lam, E.; Mortazavi, A.; Kendra, K.; Lesinski, G.B.; Mace, T.A.; Geyer, S.; Carson, W.E.; Tahiri, S.; Bhinder, A.; *et al.* A phase I study of high-dose interleukin-2 with sorafenib in patients with metastatic renal cell carcinoma and melanoma. *J. Immunother.* **2014**, *37*, 180–186.
48. Daud, A.I.; Mirza, N.; Lenox, B.; Andrews, S.; Urbas, P.; Gao, G.X.; Lee, J.H.; Sondak, V.K.; Riker, A.I.; Deconti, R.C.; *et al.* Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. *J. Clin. Oncol.* **2008**, *26*, 3235–3241.
49. Brimnes, M.K.; Vangsted, A.J.; Knudsen, L.M.; Gimsing, P.; Gang, A.O.; Johnsen, H.E.; Svane, I.M. Increased level of both $CD4^+FOXP3^+$ regulatory T cells and $CD14^+HLA\text{-}DR^-$/low myeloid-derived suppressor cells and decreased level of dendritic cells in patients with multiple myeloma. *Scand. J. Immunol.* **2010**, *72*, 540–547.
50. Brtnicky, T.; Fialova, A.; Lastovicka, J.; Rob, L.; Spisek, R. Clinical relevance of regulatory T cells monitoring in the peripheral blood of ovarian cancer patients. *Hum. Immunol.* **2014**, doi:10.1016/j.humimm.2014.12.004.
51. Ilkovitch, D.; Lopez, D.M. The liver is a site for tumor-induced myeloid-derived suppressor cell accumulation and immunosuppression. *Cancer Res.* **2009**, *69*, 5514–5521.
52. Chen, L.; Deng, H.; Lu, M.; Xu, B.; Wang, Q.; Jiang, J.; Wu, C. B7-H1 expression associates with tumor invasion and predicts patient's survival in human esophageal cancer. *Int. J. Clin. Exp. Pathol.* **2014**, *7*, 6015–6023.
53. Hasegawa, T.; Suzuki, H.; Yamaura, T.; Muto, S.; Okabe, N.; Osugi, J.; Hoshino, M.; Higuchi, M.; Ise, K.; Gotoh, M. Prognostic value of peripheral and local forkhead box P3 regulatory T cells in patients with non-small-cell lung cancer. *Mol. Clin. Oncol.* **2014**, *2*, 685–694.
54. Yukawa, T.; Shimizu, K.; Maeda, A.; Yasuda, K.; Saisho, S.; Okita, R.; Nakata, M. Cyclooxygenase-2 genetic variants influence intratumoral infiltration of Foxp3-positive regulatory T cells in non-small cell lung cancer. *Oncol. Rep.* **2015**, *33*, 74–80.
55. Yin, Y.; Cai, X.; Chen, X.; Liang, H.; Zhang, Y.; Li, J.; Wang, Z.; Chen, X.; Zhang, W.; Yokoyama, S.; *et al.* Tumor-secreted miR-214 induces regulatory T cells: A major link between immune evasion and tumor growth. *Cell Res.* **2014**, *24*, 1164–1180.
56. Lim, K.P.; Chun, N.A.; Ismail, S.M.; Abraham, M.T.; Yusoff, M.N.; Zain, R.B.; Ngeow, W.C.; Ponniah, S.; Cheong, S.C. $CD4^+CD25^{hi}CD127^{low}$ regulatory T cells are increased in oral squamous cell carcinoma patients. *PLoS One* **2014**, *9*, e103975.
57. Lunardi, S.; Jamieson, N.B.; Lim, S.Y.; Griffiths, K.L.; Carvalho-Gaspar, M.; al-Assar, O.; Yameen, S.; Carter, R.C.; McKay, C.J.; Spoletini, G.; *et al.* IP-10/CXCL10 induction in human pancreatic cancer stroma influences lymphocytes recruitment and correlates with poor survival. *Oncotarget* **2014**, *5*, 11064–11080.

58. Mirza, N.; Fishman, M.; Fricke, I.; Dunn, M.; Neuger, A.M.; Frost, T.J.; Lush, R.M.; Antonia, S.; Gabrilovich, D.I. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res.* **2006**, *66*, 9299–9307.
59. Kusmartsev, S.; Su, Z.; Heiser, A.; Dannull, J.; Eruslanov, E.; Kubler, H.; Yancey, D.; Dahm, P.; Vieweg, J. Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. *Clin. Cancer Res.* **2008**, *14*, 8270–8278.
60. Tazzari, M.; Negri, T.; Rini, F.; Vergani, B.; Huber, V.; Villa, A.; Dagrada, P.; Colombo, C.; Fiore, M.; Gronchi, A.; *et al.* Adaptive immune contexture at the tumour site and downmodulation of circulating myeloid-derived suppressor cells in the response of solitary fibrous tumour patients to anti-angiogenic therapy. *Br. J. Cancer* **2014**, *111*, 1350–1362.
61. Chu, R.; Liu, S.Y.; Vlantis, A.C.; van Hasselt, C.A.; Ng, E.K.; Fan, M.D.; Ng, S.K.; Chan, A.B.; Du, J.; Wei, W.; *et al.* Inhibition of Foxp3 in cancer cells induces apoptosis of thyroid cancer cells. *Mol. Cell. Endocrinol.* **2015**, *399*, 228–234.
62. Sayour, E.J.; McLendon, P.; McLendon, R.; de Leon, G.; Reynolds, R.; Kresak, J.; Sampson, J.H.; Mitchell, D.A. Increased proportion of FoxP3+ regulatory T cells in tumor infiltrating lymphocytes is associated with tumor recurrence and reduced survival in patients with glioblastoma. *Cancer Immunol. Immunother.* **2015**, doi:10.1007/s00262-014-1651-7.
63. Pan, P.Y.; Wang, G.X.; Yin, B.; Ozao, J.; Ku, T.; Divino, C.M.; Chen, S.H. Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. *Blood* **2008**, *111*, 219–228.
64. Liaw, Y.F.; Leung, N.W.; Chang, T.T.; Guan, R.; Tai, D.I.; Ng, K.Y.; Chien, R.N.; Dent, J.; Roman, L.; Edmundson, S.; *et al.* Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *Gastroenterology* **2000**, *119*, 172–180.
65. Kondo, Y.; Ueno, Y.; Shimosegawa, T. Immunopathogenesis of hepatitis B persistent infection: Implications for immunotherapeutic strategies. *Clin. J. Gastroenterol.* **2009**, *2*, 71–79.
66. Yang, S.H.; Lee, C.G.; Park, S.H.; Im, S.J.; Kim, Y.M.; Son, J.M.; Wang, J.S.; Yoon, S.K.; Song, M.K.; Ambrozaitis, A.; *et al.* Correlation of antiviral T-cell responses with suppression of viral rebound in chronic hepatitis B carriers: A proof-of-concept study. *Gene Ther.* **2006**, *13*, 1110–1117.
67. Kondo, Y.; Ueno, Y.; Ninomiya, M.; Tamai, K.; Tanaka, Y.; Inoue, J.; Kakazu, E.; Kobayashi, K.; Kimura, O.; Miura, M.; *et al.* Sequential immunological analysis of HBV/HCV co-infected patients during Peg-IFN/RBV therapy. *J. Gastroenterol.* **2012**, *47*, 1323–1335.
68. Franzese, O.; Kennedy, P.T.; Gehring, A.J.; Gotto, J.; Williams, R.; Maini, M.K.; Bertoletti, A. Modulation of the $CD8^+$-T-cell response by $CD4^+CD25^+$ regulatory T cells in patients with hepatitis B virus infection. *J. Virol.* **2005**, *79*, 3322–3328.
69. Stoop, J.N.; van der Molen, R.G.; Baan, C.C.; van der Laan, L.J.; Kuipers, E.J.; Kusters, J.G.; Janssen, H.L. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* **2005**, *41*, 771–778.
70. Xu, D.; Fu, J.; Jin, L.; Zhang, H.; Zhou, C.; Zou, Z.; Zhao, J.M.; Zhang, B.; Shi, M.; Ding, X.; *et al.* Circulating and liver resident $CD4^+CD25^+$ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J. Immunol.* **2006**, *177*, 739–747.

71. Stoop, J.N.; van der Molen, R.G.; Kuipers, E.J.; Kusters, J.G.; Janssen, H.L. Inhibition of viral replication reduces regulatory T cells and enhances the antiviral immune response in chronic hepatitis B. *Virology* **2007**, *361*, 141–148.
72. Yang, G.; Liu, A.; Xie, Q.; Guo, T.B.; Wan, B.; Zhou, B.; Zhang, J.Z. Association of $CD4^+CD25^+Foxp3^+$ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. *Int. Immunol.* **2007**, *19*, 133–140.
73. Otano, I.; Suarez, L.; Dotor, J.; Gonzalez-Aparicio, M.; Crettaz, J.; Olague, C.; Vales, A.; Riezu, J.I.; Larrea, E.; Borras, F.; *et al.* Modulation of regulatory T-cell activity in combination with interleukin-12 increases hepatic tolerogenicity in woodchucks with chronic hepatitis B. *Hepatology* **2012**, *56*, 474–483.
74. Stross, L.; Gunther, J.; Gasteiger, G.; Asen, T.; Graf, S.; Aichler, M.; Esposito, I.; Busch, D.H.; Knolle, P.; Sparwasser, T.; *et al.* $Foxp3^+$ regulatory T cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis B virus infection in mice. *Hepatology* **2012**, *56*, 873–883.
75. Yang, P.; Li, Q.J.; Feng, Y.; Zhang, Y.; Markowitz, G.J.; Ning, S.; Deng, Y.; Zhao, J.; Jiang, S.; Yuan, Y.; *et al.* TGF-β-miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. *Cancer Cell* **2012**, *22*, 291–303.
76. Dong, X.; Gong, Y.; Zeng, H.; Hao, Y.; Wang, X.; Hou, J.; Wang, J.; Li, J.; Zhu, Y.; Liu, H.; *et al.* Imbalance between circulating $CD4^+$ regulatory T and conventional T lymphocytes in patients with HBV-related acute-on-chronic liver failure. *Liver Int.* **2013**, *33*, 1517–1526.
77. Tang, Y.; Jiang, L.; Zheng, Y.; Ni, B.; Wu, Y. Expression of CD39 on $FoxP3^+$ T regulatory cells correlates with progression of HBV infection. *BMC Immunol.* **2012**, *13*, doi:10.1186/1471-2172-13-17.
78. Boni, C.; Penna, A.; Ogg, G.S.; Bertoletti, A.; Pilli, M.; Cavallo, C.; Cavalli, A.; Urbani, S.; Boehme, R.; Panebianco, R.; *et al.* Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: New perspectives for immune therapy. *Hepatology* **2001**, *33*, 963–971.
79. Zhang, Y.; Lian, J.Q.; Huang, C.X.; Wang, J.P.; Wei, X.; Nan, X.P.; Yu, H.T.; Jiang, L.L.; Wang, X.Q.; Zhuang, Y.; *et al.* Overexpression of Toll-like receptor 2/4 on monocytes modulates the activities of $CD4^+CD25^+$ regulatory T cells in chronic hepatitis B virus infection. *Virology* **2010**, *397*, 34–42.
80. Stoop, J.N.; Woltman, A.M.; Biesta, P.J.; Kusters, J.G.; Kuipers, E.J.; Janssen, H.L.; van der Molen, R.G. Tumor necrosis factor alpha inhibits the suppressive effect of regulatory T cells on the hepatitis B virus-specific immune response. *Hepatology* **2007**, *46*, 699–705.
81. Fisicaro, P.; Valdatta, C.; Massari, M.; Loggi, E.; Biasini, E.; Sacchelli, L.; Cavallo, M.C.; Silini, E.M.; Andreone, P.; Missale, G.; *et al.* Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology* **2010**, *138*, 682–693.
82. Franceschini, D.; Paroli, M.; Francavilla, V.; Videtta, M.; Morrone, S.; Labbadia, G.; Cerino, A.; Mondelli, M.U.; Barnaba, V. PD-L1 negatively regulates $CD4^+CD25^+Foxp3^+$ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. *J. Clin. Investig.* **2009**, *119*, 551–564.

83. Evans, A.; Riva, A.; Cooksley, H.; Phillips, S.; Puranik, S.; Nathwani, A.; Brett, S.; Chokshi, S.; Naoumov, N.V. Programmed death 1 expression during antiviral treatment of chronic hepatitis B: Impact of hepatitis B e-antigen seroconversion. *Hepatology* **2008**, *48*, 759–769.
84. Peng, G.; Li, S.; Wu, W.; Sun, Z.; Chen, Y.; Chen, Z. Circulating $CD4^+CD25^+$ regulatory T cells correlate with chronic hepatitis B infection. *Immunology* **2008**, *123*, 57–65.
85. Nan, X.P.; Zhang, Y.; Yu, H.T.; Li, Y.; Sun, R.L.; Wang, J.P.; Bai, X.F. Circulating $CD4^+CD25^{high}$ regulatory T cells and expression of PD-1 and BTLA on $CD4^+$ T cells in patients with chronic hepatitis B virus infection. *Viral Immunol.* **2010**, *23*, 63–70.
86. Nan, X.P.; Zhang, Y.; Yu, H.T.; Sun, R.L.; Peng, M.J.; Li, Y.; Su, W.J.; Lian, J.Q.; Wang, J.P.; Bai, X.F. Inhibition of viral replication downregulates $CD4^+CD25^{high}$ regulatory T cells and programmed death-ligand 1 in chronic hepatitis B. *Viral Immunol.* **2012**, *25*, 21–28.
87. Niu, Y.; Liu, H.; Yin, D.; Yi, R.; Chen, T.; Xue, H.; Zhang, S.; Lin, S.; Zhao, Y. The balance between intrahepatic IL-17^+ T cells and $Foxp3^+$ regulatory T cells plays an important role in HBV-related end-stage liver disease. *BMC Immunol.* **2011**, *12*, doi:10.1186/1471-2172-12-47.
88. Li, J.; Qiu, S.J.; She, W.M.; Wang, F.P.; Gao, H.; Li, L.; Tu, C.T.; Wang, J.Y.; Shen, X.Z.; Jiang, W. Significance of the balance between regulatory T (Treg) and T helper 17 (Th17) cells during hepatitis B virus related liver fibrosis. *PLoS One* **2012**, *7*, e39307.
89. Xue-Song, L.; Cheng-Zhong, L.; Ying, Z.; Mo-Bin, W. Changes of Treg and Th17 cells balance in the development of acute and chronic hepatitis B virus infection. *BMC Gastroenterol.* **2012**, *12*, doi:10.1186/1471-230X-12-43.
90. Li, J.; Shi, J.; Ren, W.; Wu, W.; Chen, Z. Regulatory role of $CD4^+CD25^+Foxp3^+$ regulatory T cells on IL-17-secreting T cells in chronic hepatitis B patients. *Dig. Dis. Sci.* **2014**, *59*, 1475–1483.
91. Sprengers, D.; Stoop, J.N.; Binda, R.S.; Kusters, J.G.; Haagmans, B.L.; Carotenuto, P.; Artsen, A.; van der Molen, R.G.; Janssen, H.L. Induction of regulatory T-cells and interleukin-10-producing cells in non-responders to pegylated interferon-alpha therapy for chronic hepatitis B. *Antivir. Ther.* **2007**, *12*, 1087–1096.
92. Aalaei-Andabili, S.H.; Alavian, S.M. Regulatory T cells are the most important determinant factor of hepatitis B infection prognosis: A systematic review and meta-analysis. *Vaccine* **2012**, *30*, 5595–5602.
93. Ormandy, L.A.; Hillemann, T.; Wedemeyer, H.; Manns, M.P.; Greten, T.F.; Korangy, F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res.* **2005**, *65*, 2457–2464.
94. Yang, X.H.; Yamagiwa, S.; Ichida, T.; Matsuda, Y.; Sugahara, S.; Watanabe, H.; Sato, Y.; Abo, T.; Horwitz, D.A.; Aoyagi, Y. Increase of $CD4^+CD25^+$ regulatory T-cells in the liver of patients with hepatocellular carcinoma. *J. Hepatol.* **2006**, *45*, 254–262.
95. Cao, M.; Cabrera, R.; Xu, Y.; Firpi, R.; Zhu, H.; Liu, C.; Nelson, D.R. Hepatocellular carcinoma cell supernatants increase expansion and function of $CD4^+$ $CD25^+$ regulatory T cells. *Lab. Investig.* **2007**, *87*, 582–590.
96. Fu, J.; Xu, D.; Liu, Z.; Shi, M.; Zhao, P.; Fu, B.; Zhang, Z.; Yang, H.; Zhang, H.; Zhou, C.; *et al.* Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* **2007**, *132*, 2328–2339.

97. Fu, J.; Zhang, Z.; Zhou, L.; Qi, Z.; Xing, S.; Lv, J.; Shi, J.; Fu, B.; Liu, Z.; Zhang, J.Y.; *et al.* Impairment of $CD4^+$ cytotoxic T cells predicts poor survival and high recurrence rates in patients with hepatocellular carcinoma. *Hepatology* **2013**, *58*, 139–149.
98. Takata, Y.; Nakamoto, Y.; Nakada, A.; Terashima, T.; Arihara, F.; Kitahara, M.; Kakinoki, K.; Arai, K.; Yamashita, T.; Sakai, Y.; *et al.* Frequency of $CD45RO^+$ subset in $CD4^+CD25^{high}$ regulatory T cells associated with progression of hepatocellular carcinoma. *Cancer Lett.* **2011**, *307*, 165–173.
99. Zhu, J.; Feng, A.; Sun, J.; Jiang, Z.; Zhang, G.; Wang, K.; Hu, S.; Qu, X. Increased $CD4^+CD69^+CD25^-$ T cells in patients with hepatocellular carcinoma are associated with tumor progression. *J. Gastroenterol. Hepatol.* **2011**, *26*, 1519–1526.
100. Zhang, H.H.; Mei, M.H.; Fei, R.; Liu, F.; Wang, J.H.; Liao, W.J.; Qin, L.L.; Wei, L.; Chen, H.S. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. *J. Viral Hepat.* **2010**, *17*, 34–43.
101. Wang, Q.; Yu, T.; Yuan, Y.; Zhuang, H.; Wang, Z.; Liu, X.; Feng, M. Sorafenib reduces hepatic infiltrated regulatory T cells in hepatocellular carcinoma patients by suppressing TGF-β signal. *J. Surg. Oncol.* **2013**, *107*, 422–427.

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Review Article

New molecularly targeted therapies against advanced hepatocellular carcinoma: From molecular pathogenesis to clinical trials and future directions

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Hepatocellular carcinoma (HCC) can be lethal due to its aggressive course and lack of effective systemic therapies for advanced disease. Sorafenib is the only systemic therapy that has demonstrated an overall survival benefit in patients with advanced HCC, and new agents for treatment of advanced HCC are needed. The multiple pathways involved in HCC oncogenesis, proliferation and survival provide many opportunities for the development of molecularly targeted therapies. Molecular targets of interest have expanded from angiogenesis to cancer cell-directed oncogenic signaling pathways for treatment of advanced HCC. Agents targeting vascular endothelial growth factor receptor, epidermal growth factor receptor, fibroblast growth factor receptor, platelet-derived growth factor receptor, c-mesenchymal-epithelial transition factor-1 and mammalian target of rapamycin signaling have been actively explored. This article focuses on the evaluation of molecular agents targeting pathogenic HCC and provides a review of recently completed phase III drug studies (e.g. involving sorafenib, sunitinib, brivanib, linifanib, erlotinib, everolimus, ramucirumab or orantinib) and ongoing drug studies (e.g. involving lenvatinib, regorafenib, tivantinib or cabozantinib) of molecularly targeted agents in advanced HCC, including a brief description of the biologic rationale behind these agents.

Key words: clinical trials, hepatocellular carcinoma, molecularly targeted therapy, novel agents, sorafenib

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the sixth most common cancer and the third most common cause of cancer-related deaths worldwide.[1] Because a considerable number of patients are diagnosed when the disease becomes advanced, only approximately a third of all HCC patients are eligible for potentially curative treatments, such as resection or transplantation.[2] Surgical resection or transplantation provides good survival rates (i.e. beyond 65% at 5 years).[3] Unfortunately, the prognosis for patients with advanced stage HCC (Barcelona Cancer Liver Clinic [BCLC] stage C) is extremely poor, with a median overall survival (OS) of 6.6 months.[4] In advanced cases, only one systemic therapy is effective: the multikinase inhibitor sorafenib, which was approved by the U.S. Food and Drug Administration and which represented a breakthrough in the management of the disease.[5,6] However, the median life expectancy of patients with HCC on sorafenib is only 1 year, indicating the clear need to improve their outcomes. Hepatocarcinogenesis is a complex multistep process involving a number of genetic and epigenetic alterations,[7,8] our knowledge of several key molecular pathways implicated in HCC pathogenesis has revealed potential targets for therapeutic interventions, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), RAS/RAF/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways. This review will examine our current understanding of these pathways as well as the efficacy and safety data pertaining to the most promising

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molecularly targeted agents beyond sorafenib. In this article, we first describe the pathogenesis of HCC and then provide an update on the recent data on clinical trials using molecularly targeted agents.

PATHOGENESIS OF HCC

HEPATOCELLULAR CARCINOMA IS a hypervascular tumor, and the central role of angiogenesis in its initiation, growth and subsequent dissemination to other tissues is well recognized. Angiogenesis in HCC is mediated by a complex network of growth factors, acting on both tumor cells and endothelial cells. The most widely recognized angiogenic factors are VEGF, FGF and PDGF.[9] These activate receptor tyrosine kinases (RTK) and the RAS/RAF/MEK/ERK pathway and the PI3K pathway in endothelial cells (Fig. 1).[10,11] VEGF and its receptors play a major role in tumor angiogenesis. In fact, VEGF is a potent permeability factor that promotes cell migration during invasion and acts as an endothelial growth factor that stimulates endothelial cell proliferation, inducing the budding of new blood vessels around the growing tumor masses. In human specimens and serum, increased expression of VEGF correlates with aggressive behavior of HCC and poor prognosis.[12] FGF and its family of receptors has also been implicated in HCC growth, invasion and angiogenesis.[13] While VEGF is the main driver of tumor angiogenesis, there is crosstalk between VEGF and FGF signaling in angiogenesis.[14] The upregulation of FGF has been suggested to mediate resistance to anti-VEGF receptor (VEGFR) therapy. The great majority of the HCC cases have overexpression of at least one FGF and/or FGF receptor (FGFR).[10] Hence, blocking the FGFR is another potentially important approach for the treatment of HCC. PDGF is involved in the development of immature tumor vessels,[15] while angiopoietins exert their action via stabilization of vessels by recruiting surrounding pericytes and smooth

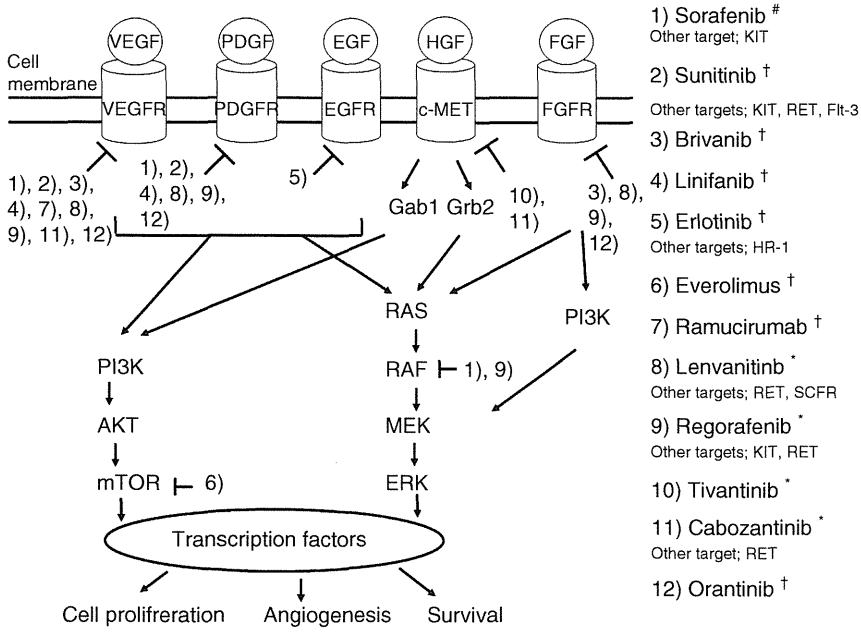

Figure 1 Schematic diagram of key molecular targets and targeted agents in hepatocellular carcinoma. #Approved globally in patients with advanced hepatocellular carcinoma (HCC). †Completed studies of phase III of molecularly targeted agents in advanced HCC. *Ongoing studies of phase III of molecularly targeted agents in advanced HCC. c-MET, c-mesenchymal-epithelial transition factor-1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; Flt-3, Fms-like tyrosine receptor kinase-3; Gab1, GRB2-associated binding protein 1; Grb2, growth factor receptor bound protein 2; HER-1, human epidermal growth factor receptor-1; HGF, hepatocyte growth factor; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; RET, rearranged during transfection; SCFR, stem cell growth factor receptor kit; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

muscle cells.[16] PDGF is involved in fibrogenesis, angiogenesis and tumorigenesis.[17,18] PDGF expression is upregulated in the early stages of chronic hepatitis, suggesting its association with the development of fibrosis in chronic hepatitis C.[19] From a therapeutic point of view, inhibition of these targets has been shown to diminish the vascularity of tumors in preclinical studies.

Several intracellular signaling pathways are involved in HCC pathogenesis; the most studied are the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways. The PI3K/AKT/mTOR axis is involved in multiple cellular processes, including survival and proliferation.[20] This pathway mediates its effects through activation of various tyrosine kinase receptors, such as VEGFR, EGFR and PDGFR, which in turn recruit and activate PI3K. The activation of PI3K will lead to a cascade of activation of downstream effectors, leading to activation of mTOR (Fig. 1). The activation of the mTOR pathway in HCC is associated with aggressive tumor behavior and decreased survival, which supports the efforts to target this pathway for therapeutic interventions.[21] RAS/RAF/MEK/ERK signaling regulates many important cellular processes, such as proliferation, differentiation, angiogenesis, survival and cell adhesion.[22] Importantly, the RAS/RAF/MEK/ERK pathway is constitutively activated in HCC.[23]

Apart from these major signal pathways in the pathogenesis of HCC, the hepatocyte growth factor (HGF)/c-mesenchymal-epithelial transition factor-1 (c-MET) pathway is involved in tumor growth, invasion and angiogenesis in various types of cancer.[24] c-MET is a tyrosine kinase receptor, with its ligand, HGF.[25] HGF-induced activation of c-MET ultimately leads to the activation of downstream effector molecules, including PI3K and ERK (Fig. 1).[26] Expression of the c-MET receptor protein is present in human HCC samples[27–29] and has been shown to be a poor prognostic factor in patients with HCC. Therefore, therapeutics aimed at the c-MET receptor is a rational approach for HCC.

RESULTS OF PHASE III STUDIES

STUDIES ARE INVESTIGATING various agents for HCC, most of which target the previously described VEGF axis, FGF, PDGF, RAS/RAF/ERK and mTOR signaling pathways (Fig. 1). We describe these molecularly targeted agents and completed phase III trials. We also provide information on why phase III pivotal consecutive randomized controlled trials (RCT) in HCC did not meet the primary end-points (Table 1). Seven phase III trials reported negative results for first-line therapy (e.g. with sunitinib, brivanib, linifanib or erlotinib) and second-line therapy (e.g. with brivanib, everolimus or ramucirumab). Five of these studies were designed to test for superiority (i.e. study of SUN 1170, SEARCH, BRISK-PS, EVOLVE-1, REACH), and two of these studies were designed to test for non-inferiority (i.e. study of BRISK-FL, 0100953) with a primary end-point of OS.

Sorafenib

Sorafenib is a multikinase inhibitor that inhibits serine/threonine kinases (BRaf and CRaf and VEGFR-1, -2 and -3), PDGFR-α and -β, and the stem cell factor receptor, c-kit. In the Sorafenib HCC Assessment Randomized Protocol (SHARP) study,[5] a double-blind RCT with a primary end-point of OS, sorafenib significantly increased survival times of patients with HCC from 7.9 to 10.7 months (hazard ratio [HR], 0.69; 95% confidence interval [CI], 0.55–0.87; $P = 0.001$). Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 97% and 3%, respectively, while that with BCLC stage B and C disease was 17% and 83%, respectively. Sorafenib was the first systemic therapy to demonstrate a significant improvement in OS in patients with advanced HCC, and its subsequent approval represented a major breakthrough in the treatment of advanced HCC. A parallel phase III study was conducted in the Asia–Pacific region. Median OS was 6.5 months in the sorafenib arm and 4.2 months in the placebo arm (HR, 0.68; 95% CI, 0.50–0.93; $P = 0.014$).[6] Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 97% and 3%, respectively, while that with BCLC stage B and C disease was 5% and 95%, respectively. Similar toxicity profiles were seen in both studies; sorafenib treatment was associated with increased rates of diarrhea, weight loss, hand–foot skin reaction and hypophosphatemia. Sorafenib is the first and only agent to demonstrate an OS benefit and to be approved by regulators globally in patients with advanced HCC.

Sunitinib

Sunitinib is another multikinase inhibitor with broad activity, inhibiting all VEGFR and PDGFR, c-kit, Fms-like tyrosine receptor kinase (Flt)3 and rearranged during transfection (RET). Sunitinib was evaluated against sorafenib in a large phase III trial.[30] All patients had Child–Pugh liver function class A disease, and the proportion of patients with BCLC stage B and C disease was 15% and 85%, respectively. Median time to progression (TTP) for sunitinib and sorafenib was 4.1 and 3.8

Table 1 Results of completed phase III trials of molecularly targeted therapies in HCC

Drug	Main target	Design (trial)	TTP/PFS (months), HR, 95% CI	OS (months), HR, 95% CI
First-line advanced HCC				
Sorafenib	RAF, VEGFR, PDGFR, c-KIT	Sorafenib vs placebo (SHARP)	4.9 vs 4.1; $P = 0.77$; HR, 0.58; 95% CI, 0.45–0.74	10.7 vs 7.9; $P < 0.001$; HR, 0.69; 95% CI, 0.55–0.87
		Sorafenib vs placebo (Asia–Pacific)	2.8 vs 1.4; $P < 0.001$; HR, 0.57; 95% CI, 0.42–0.79	6.5 vs 4.2; $P = 0.014$; HR, 0.68; 95% CI, 0.50–0.93
Sunitinib	VEGFR, PDGFR, KIT, RET, Flt-3	Sunitinib vs sorafenib (SUN 1170)	4.1 vs 3.8; $P = 0.169$; HR, 1.13; 95% CI, 0.98–1.31	7.9 vs 10.2; $P = 0.0019$; HR, 1.30; 95% CI, 1.13–1.50
Brivanib	FGFR, VEGFR	Brivanib vs sorafenib (BRISK-FL)	4.2 vs 4.1; $P = 0.853$; HR, 1.01; 95% CI, 0.88–1.16	9.5 vs 9.9; $P = 0.373$; HR, 1.06; 95% CI, 0.93–1.22
Linifanib	VEGFR, PDGFR	Linifanib vs sorafenib (0100953)	5.4 vs 4.0; $P = 0.001$; HR, 0.76; 95% CI, 0.64–0.90	9.1 vs 9.8; $P =$ NS; HR, 1.05; 95% CI, 0.90–1.22
Erlotinib	EGFR, HER-1	Erlotinib + sorafenib vs placebo + sorafenib (SEARCH)	3.2 vs 4.0; $P = 0.91$; HR, 1.13; 95% CI, 0.94–1.36	9.5 vs 8.5; $P = 0.2$; HR, 0.92; 95% CI, 0.78–1.1
Second-line advanced HCC				
Brivanib	FGFR, VEGFR	Brivanib vs placebo (BRISK-PS)	4.2 vs 2.7; $P < 0.001$ HR, 0.56; 95% CI, 0.42–0.78	9.4 vs 8.2; $P = 0.331$; HR, 0.89; 95% CI, 0.69–1.15
Everolimus	mTOR	Everolimus vs placebo (EVOLVE-1)	3.0 vs 2.6; HR, 0.93; 95% CI, 0.75–1.15	7.6 vs 7.3; $P = 0.68$; HR, 1.27; 95% CI, 0.86–1.27
Ramucirumab	VEGFR	Ramucirumab vs placebo (REACH)	2.8 vs 2.1; $P < 0.001$; HR, 0.63; 95% CI, 0.52–0.75†	9.2 vs 7.6; $P = 0.14$; HR, 0.87; 95% CI, 0.72–1.05

†Progression-free survival.
CI, confidence interval; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; HER-1, human epidermal growth factor receptor-1; HR, hazard ratio; mTOR, mammalian target of rapamycin; NS, not significant; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; RET, rearranged during transfection, Flt-3, Fms-like tyrosine receptor kinase-3; TTP, time to progression; VEGFR, vascular endothelial growth factor receptor.

months, respectively ($P = 0.169$); however, median OS for sunitinib and sorafenib was 7.9 and 10.2 months (HR, 1.30; 95% CI, 1.13–1.50; $P = 0.0019$), respectively. The decision was based on a higher incidence of significant toxicities (including grade 3/4 thrombocytopenia [30%], neutropenia [25%] and hemorrhagic events [12%]) in the sunitinib arm and the futility of showing either superiority or non-inferiority in OS when compared with sorafenib. This trial was stopped prematurely after inferior outcomes were noted in the sunitinib arm.

Brivanib

Brivanib is a dual inhibitor of VEGFR and FGFR, both of which are implicated in the pathogenesis of HCC.[31] Two randomized phase III clinical trials were conducted to assess the use of brivanib in the first-line (BRISK-FL) and second-line (BRISK-PS) settings. BRISK-FL was a head-to-head randomized phase III clinical trial comparing brivanib with sorafenib as the first-line therapy in patients with unresectable HCC. Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 92% and 8%, respectively, while that with BCLC stage B and C disease was 22% and 78%, respectively. The brivanib arm failed to achieve a non-inferior median OS, with 9.5 months for brivanib and 9.9 months for sorafenib (HR, 1.06; 95% CI, 0.93–1.22; $P = 0.373$). There was also no difference in TTP between brivanib and sorafenib (4.2 vs 4.1 months; HR, 1.01; 95% CI, 0.88–1.16; $P = 0.853$).[31] The study did not meet its primary OS objective based upon a non-inferiority statistical design. In the second-line setting, BRISK-PS compared brivanib with placebo in patients who were refractory or intolerant to first-line treatment with sorafenib. Although TTP was significantly longer in the brivanib arm than with placebo (4.2 vs 2.7 months; HR, 0.56; 95% CI, 0.42–0.78; $P < 0.001$), the primary end-point of the study was not met, with a median OS for brivanib and placebo of 9.4 and 8.2 months, respectively (HR, 0.89; 95% CI, 0.69–1.15; $P = 0.331$).[32] The most common grade 3/4 adverse events (AE) were hypertension (19%), hyponatremia (18%), fatigue (15%) and decreased appetite (12%).

Linifanib

Linifanib is an oral tyrosine kinase inhibitor (TKI) with selective activity against VEGFR and PDGFR. Linifanib was compared with sorafenib as first-line therapy in a non-inferiority phase III trial.[33] Enrolled patients were those with a histological and cytological diagnosis of unresectable HCC and Child–Pugh liver function class A. TTP with linifanib was significantly improved when compared with sorafenib (5.4 vs 4.0 months; HR, 0.76; 95% CI, 0.64–0.90; $P = 0.001$). However, median OS was 9.1 months with linifanib and 9.8 months with sorafenib (HR, 1.05; 95% CI, 0.90–1.22). Linifanib was less well tolerated than sorafenib, with significantly increased discontinuations and dose reductions/interruptions because of AE.

Erlotinib

Erlotinib is an orally active, potent selective inhibitor of the EGFR/human epidermal growth factor receptor-1-related tyrosine kinase enzyme. In the phase III SEARCH trial, advanced HCC patients were randomized to sorafenib plus either erlotinib or placebo.[34] Inclusion criteria were a histological and cytological diagnosis of unresectable HCC and Child–Pugh liver function class A. Median OS was 9.5 months with sorafenib plus erlotinib and 8.5 months with sorafenib (HR, 0.92; 95% CI, 0.78–1.1; $P = 0.2$). This result failed the prespecified boundaries for non-inferiority. TTP was 3.2 months with sorafenib plus erlotinib and 4.0 months with sorafenib (HR, 1.13; 95% CI, 0.94–1.36; $P = 0.91$).

Everolimus

The mTOR inhibitor, everolimus, has demonstrated antitumor activity in several malignancies. A phase III study comparing everolimus with placebo (EVOLVE-1) in patients who have failed or become intolerant to sorafenib has recently been completed. All patients had Child–Pugh liver function class A, and the proportion of patients with BCLC stage B and C disease was 14% and 86%, respectively. There were no significant difference in TTP between everolimus (3.0 months) and placebo (2.6 months) (HR, 0.93; 95% CI, 0.75–1.15). Furthermore, no significant difference in OS was seen between everolimus (7.6 months) and placebo (7.3 months) (HR, 1.05; 95% CI, 0.86–1.27; $P = 0.68$). The most common grade 3/4 AE for everolimus were anemia (7.8%), asthenia (7.8%) and decreased appetite (6.1%). No patients experienced hepatitis C viral flare. The EVOLVE-1 study failed to reach its primary end-point of extending OS with everolimus.[35]

Ramucirumab

Ramucirumab is a recombinant humanized antibody that specifically targets the extracellular domain of VEGFR-2. A phase II study of 42 patients with advanced HCC and primarily well-preserved liver function showed that first-line ramucirumab monotherapy produced a disease control rate of 69%. The median progression-free survival (PFS) was 4.0 months and

median OS was 12.0 months, respectively. Grade 3/4 toxicities included gastrointestinal bleeding (7%), hypertension (12%) and fatigue (10%). These findings prompted the initiation of the phase III RCT (REACH) comparing ramucirumab versus placebo in patients who failed or were intolerant to sorafenib (NCT01140347).[36] Eligible patients had advanced HCC, stage BCLC C or B disease that was refractory or not amenable to locoregional therapy, and Child-Pugh liver function class A. However, according to the preliminary results released at European Society for Medical Oncology Congress in 2014, ramucirumab failed to demonstrate superiority in terms of OS when compared with placebo. The OS HR was 0.866 (95% CI, 0.717–1.046; $P = 0.1391$); median OS was 9.2 months for ramucirumab versus 7.6 months for placebo. Median PFS with ramucirumab and placebo was 2.8 and 2.1 months, respectively (HR, 0.63, 95% CI, 0.52–0.75; $P < 0.0001$).[37]

ONGOING PHASE III CLINICAL TRIALS

IN ADDITION TO the antiangiogenic multi-targeted TKI, there is a growing number of biologics that target different molecular pathways, such as c-MET. Some of these treatments act on elements of intracellular signaling pathways. A number of agents have shown promising preliminary data for HCC. We also comment on ongoing phase III pivotal trials (Table 2). The inclusion criterion of all four phase III studies was Child-Pugh liver function class A disease.

Lenvatinib

Lenvatinib is an oral multi-tyrosine kinase inhibitor that targets VEGFR-1–3, FGFR-1–3, RET, mast/stem cell growth factor receptor kit and PDGFR.[38] A phase I/II trial of lenvatinib in patients with advanced HCC and Child-Pugh score A liver function status showed a median OS of 18.7 months (95% CI, 12.8–25.1) and a median TTP of 7.4 months (95% CI, 5.5–9.4). Based on these results, a phase III trial was designed to compare the safety and efficacy of lenvatinib versus sorafenib in patients with unresectable or advanced HCC and Child-Pugh A liver status (NCT01761266).[39] Subjects were categorized as stage B (not applicable for transarterial chemoembolization [TACE]) or stage C based on the BCLC staging system.

Regorafenib

Regorafenib is a multikinase inhibitor that targets kinases involved in angiogenesis (e.g. VEGFR-1–3), oncogenesis (e.g. c-kit, RET and BRAF) and the tumor microenvironment (e.g. PDGFR and FGFR).[40] Regorafenib (160 mg/day) was tested in an uncontrolled phase II study in patients with advanced HCC after failure of prior sorafenib therapy (RESORCE).[41] Median TTP was 4.3 months and median OS was 13.8 months. The most common grade 3/4 AE included fatigue (17%), hand-foot skin reaction (14%) and diarrhea (6%). Based on this data, a phase III RCT in the second-line setting is under development (NCT01774344). Inclusion criteria were BCLC stage B or C disease, and failure to receive prior treatment with sorafenib.

Tivantinib

Tivantinib is a selective inhibitor of c-MET.[42] In a randomized phase II trial comparing the use of tivantinib

Table 2 List of ongoing phase III trials of novel targeted therapy for HCC

Drug	Main target	Design (trial)	Status	NCT number
1st line				
Lenvatinib	VEGFR, PDGFR, FGFR, RET, SCFR	Lenvatinib vs sorafenib (E7080)	Recruiting	NCT01761266
2nd line				
Regorafenib	VEGFR, PDGFR, BRAF, FGFR, KIT, RET	Regorafenib vs placebo (RESORCE)	Recruiting	NCT01774344
Tivantinib	c-MET	Tivantinib vs placebo in subjects with c-MET overexpressing (JET-HCC)	Recruiting	NCT01755767
Cabozantinib	c-MET, VEGFR, RET	Cabozantinib vs placebo (CELESTIAL)	Recruiting	NCT01908426

c-MET, c-mesenchymal-epithelial transition factor-1; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection; SCFR, stem cell growth factor receptor kit; VEGFR, vascular endothelial growth factor receptor.

Table 3 Results of completed phase III trials of molecularly targeted therapy in combination with TACE for HCC

Drug	Main target	Design	TTP (months, HR, 95% CI)	OS (months)
Sorafenib	RAF, VEGFR, PDGFR, c-KIT	TACE + sorafenib vs TACE + placebo	5.4 vs 3.7; $P = 0.252$; HR, 0.87; 95% CI, 0.70–1.09	29.7 vs NE; $P = 0.790$; HR, 1.06; 95% CI, 0.69–1.64
Brivanib	FGFR, VEGFR	TACE + brivanib vs TACE + placebo	12.0 vs 10.9; $P = 0.62$; HR, 0.94; 95% CI, 0.72–1.22	26.4 vs 26.1; $P = 0.53$; HR, 0.90; 95% CI, 0.66–1.23
Orantinib	VEGFR, PDGFR, FGFR	TACE + orantinib vs TACE + placebo	†	†

†Full data have not yet been reported at November 2014.
CI, confidence interval; HCC, hepatocellular carcinoma; FGFR, fibroblast growth factor receptor; HR, hazard ratio; NE, not estimable due to immaturity of data; OS, overall survival; PDGFR, platelet-derived growth factor receptor; TTP, time to progression; VEGFR, vascular endothelial growth factor receptor.

versus placebo as second-line treatment, the overall analysis showed a marginal but significant improvement in TTP in tivantinib over placebo (1.6 vs 1.4 months; HR, 0.64; 95% CI, 0.43–0.94; $P = 0.04$). A preplanned analysis of patients whose tumors demonstrated overexpression of MET by immunohistochemistry revealed a more notable improvement in TTP, with 2.7 months in the MET-high tivantinib subset versus 1.4 months in the MET-high placebo subset (HR, 0.43; 95% CI, 0.19–0.97; $P = 0.03$). Median OS was 7.2 months for patients with MET-high tumors who received tivantinib versus 3.8 months for MET-high patients who received placebo (HR, 0.38, 95% CI, 0.18–0.81; $P = 0.01$).[43] The most common grade 3/4 AE in the tivantinib group were neutropenia and anemia; severe neutropenia rates were higher prior to mandated dose reduction. Currently, a phase III study is underway to compare tivantinib versus placebo in subjects with c-MET-overexpressing HCC who have failed one prior systemic therapy (NCT01755767).

Cabozantinib

Cabozantinib, a multikinase inhibitor that inhibits MET, VEGFR-2 and RET, was studied in a phase II trial of HCC patients who had received at most one prior systemic therapy.[44] Impressive efficacy was observed; the PFS was 4.4 months while the median OS was 15.1 months in the cabozantinib arm.[45] A phase III clinical trial testing the efficacy of cabozantinib in the second-line setting is planned (NCT01908426).

Combination therapy

With regard to molecularly targeted agents combined with other treatments, surgical resection and local ablation are curative therapies for BCLC stage A, whereas TACE is used for the management of patients of BCLC stage B. Hepatic arterial infusion chemotherapy (HAIC) is used for the management of patients of BCLC stage B to C. In this article, we focused mainly BCLC stage B to C. Tables 3 and 4 summarizes data regarding the use of molecularly targeted agents combined with TACE or HAIC.

The high rate of HCC recurrence after TACE may be due to its enhancement of angiogenesis and upregulation of VEGF and PDGFR expression, resulting in the formation of rich vascular beds in residual tumors.[46] Administration of an antiangiogenic agents with TACE may block angiogenesis and may therefore lengthen time to recurrence and improve survival.

A phase III study of sorafenib in combination with TACE versus TACE alone performed in Japan and Korea likewise did not demonstrate any benefit with the combination (TTP; sorafenib vs placebo [5.4 vs 3.7 months, HR, 0.87; 95% CI, 0.70–1.09; $P = 0.252$]; OS sorafenib vs placebo; 29.7 months vs not estimable due to immaturity of data [HR, 1.06; 95% CI, 0.69–1.64;

Table 4 List of ongoing phase III trials of therapy in combination with TACE or HAIC for HCC

Drug	Design (trial)	Status	NCT number
Sorafenib	TACE + sorafenib vs TACE + placebo	Recruiting	NCT01004978
Sorafenib	TACE + sorafenib vs TACE + placebo	Recruiting	NCT01324076
Sunitinib	TACE + sunitinib vs TACE + placebo	Recruiting	NCT01164202
Sorafenib	HAIC + sorafenib vs sorafenib	Recruiting	NCT01214343

HAIC, hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.