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Cellular Immune Suppressor Mechanisms in Patients with Hepatocellular Carcinoma

Fei Zhao, Firouzeh Korangy, and Tim F. Greten

Gastrointestinal Malignancy Section, Medical Oncology Branch, Center for Cancer Research, National Cancer Institute

Abstract

Hepatocellular carcinoma (HCC) has been shown to induce several immune suppressor mechanisms in patients. Our laboratory has been investigating different cellular mechanisms of immune suppression in patients with HCC. These suppressor mechanisms range from CD4⁺ regulatory T cells, functionally impaired dendritic cells, neutrophils and monocytes to myeloid derived suppressor cells. *In vitro* as well as *in vivo* studies have demonstrated that abrogation of the suppressor cells enhances or unmasks tumor specific anti-tumor immune responses. We have performed literature search for immune suppressor cells in HCC and here we provide a comprehensive summary of the latest studies in this field.

Keywords

immunotherapy; tolerance; cancer; liver

With the recent approval of anti-CTLA-4 immunoglobulin as an anti-cancer drug, immunotherapy has gained a lot of interest as a new treatment option for patients with cancer including hepatocellular carcinoma (HCC). In fact, HCC represents an ideal candidate for potential immunotherapeutic approaches for a number of different reasons:

- i. HBV vaccination has been shown to protect from the development of HCC.
- ii. Type-I interferons have shown promising results for the treatment of HCC.
- iii. A number of correlative studies indicate a relation between spontaneous immune responses and patients outcome.

We and others have previously demonstrated that spontaneous tumor-specific immune responses occur frequently in HCC patients. Both humoral and cellular tumor specific immune responses to HCC can be detected [1-5]. Surprisingly, our data indicated that spontaneous immune responses in HCC patients occur at similar frequencies to the immune responses to other tumors which are more immunogenic such as melanoma. Simultaneous NY-ESO mRNA and antibody analysis for expression of NY-ESO in tumors and anti-NY-ESO antibody analysis in serum from patients with HCC revealed that at least fifty percent of HCC patients developed NY-ESO specific antibody responses even in the absence of specific interventions aiming to prime immune responses (Figure 1). Therefore, if tumor specific immune responses can already be detected without specific vaccination approaches, this clearly questions the rationale for a vaccine approach aiming to increase tumor specific immune responses [1,5-8]. This data also suggests that tumor specific cellular immune

responses are potentially overshadowed by different suppressor mechanisms disabling effective anti-tumor immunity. In this article, we review and summarize current knowledge on cellular suppressor mechanisms in patients with HCC (Figure 2).

CD4⁺CD25⁺ regulatory T cells

CD4⁺CD25⁺ regulatory T cells (Tregs) are a minor but functionally unique population of T cells, which maintain immune homeostasis in immune tolerance and the control of autoimmunity. Absence of CD4⁺CD25⁺ Tregs is associated with severe signs of autoimmunity, which can be found both in mice and human [9,10]. While *in vitro* Tregs can inhibit immune responses mediated by both CD4⁺ and CD8⁺ effector T cells by a contact-dependent and cytokine-independent mechanism [11-13], the mechanism of immune suppression is much more complex *in vivo* [14,15]. Forkhead or winged helix family of transcription factor P3 (FOXP3) is not only essential for Treg development, but also remains the best marker to identify these cells. However, a number of studies have shown that activation of human non-Tregs can also lead to expression of Foxp3 *in vitro*, so that this marker needs to be used with caution [16]. Alternatively, it has been suggested that analysis of Foxp3 methylation status can be used to determine the presence of Tregs in humans [17].

Multiple investigations have demonstrated the pivotal role of Tregs in tumor immunology and their ability to suppress anti-tumor immune responses. Accordingly, targeting Tregs has been shown to boost anti-tumor immunity. Involvement of CD4⁺CD25⁺ Tregs in human cancer has been observed in peripheral blood and tumor tissues from patients with several types of cancer [18-20]. We and others have been able to demonstrate that Tregs are increased in peripheral blood and tumor infiltrating lymphocytes of patients with HCC [21-23].

While initial investigations have only demonstrated an increase in Treg frequencies in patients with HCC, follow-up studies have explored a potential correlation with disease progression and patients' outcome [24,25]. One study demonstrated that an up-regulation of Tregs was associated with a significantly reduced CD8⁺ T cell infiltration of tumors [26]. A correlation between poor survival and increase in Tregs was also shown in the same report and supported by others [27,28]. Finally, patients with advanced HCC had a higher percentage of intra-hepatic CD8⁺Foxp3 regulatory T cells than in patients with early disease suggesting that CD8⁺Foxp3⁺ regulatory T cells also represent another immune escape mechanism [29].

Since Tregs are increased in patients with HCC and correlate with worse outcome, we examined whether Tregs also suppress tumor-specific T cell responses in patients with HCC. Indeed, we have shown that *in vitro* depletion of Tregs unmasked AFP specific immune responses in PBMC isolated from patients with HCC. Based on this *in vitro* observation, we performed a clinical trial targeting Tregs in patients with HCC. Patients were treated with low dose cyclophosphamide, which had been shown in mice to target Tregs. While the number of patients treated in this study was too small to draw any definite conclusions the results demonstrated that the frequency of Tregs in peripheral blood can be temporarily reduced by low dose cyclophosphamide treatment [14,30].

CD14⁺HLA-DR^{low/neg} myeloid derived suppressor cells (MDSC)

Myeloid derived suppressor cells (MDSC) represent a heterogeneous population of cells that consists of myeloid progenitor cells and immature myeloid cells (IMCs) [15,16,31,32]. Natural suppressor cells (the initial name for MDSC) were already described more than 25 years ago in patients with cancer [17,33]. Murine MDSCs are characterized by coexpression of Gr-1 and CD11b. CD11b⁺Gr-1⁺ MDSC represent approximately 2 to 4 % of all nucleated

splenocytes, but can increase up to 50% in tumor bearing mice [18-20,34,35]. These cells are a mixture of immature myeloid cells, immature granulocytes, monocytes, macrophages, dendritic cells and myeloid progenitor cells. MDSCs can be further subdivided into two major groups, granulocytic MDSC (CD11b⁺Gr-1^{high}) and monocytic MDSC (CD11b⁺Gr-1^{low}). Since MDSC are such heterogeneous population, efforts are ongoing to identify more markers to distinguish them better. Our laboratory has identified another marker, CD49d, to further characterize murine MDSC. In the same report we have shown that monocytic CD11b⁺CD49d⁺ MDSC are more potent suppressors of antigen-specific T cells *in vitro* than CD11b⁺CD49d⁻ granulocytic MDSC and suppress T cell responses through an NO mediated mechanism [36].

In humans, MDSC are also poorly characterized, but are also divided into monocytic and granulocytic populations. We have identified human monocytic CD14⁺HLA-DR^{low/neg} MDSC in patients with HCC [37] and described an increase in the frequency of CD14⁺HLA-DR^{low/neg} MDSC in peripheral blood and ascites in these patients. CD14⁺HLA-DR^{low/neg} MDSC failed to induce proliferative T cell responses and did not mature into dendritic cells *in vitro*. Apart from their ability to suppress non-specific T cell responses, MDSC also masked AFP-specific T cell responses [37].

In order to better understand the biology and the clinical relevance of human MDSC better, we examined the interaction of MDSC with other immune cells in more detail. Natural killer (NK) cells represent an important cell type in the context of HCC. NK cells are impaired in function in HCC patients [38]. We have demonstrated that MDSC are potent suppressors of NK cells in patients with HCC [39]. In addition, we showed that human MDSC induced a T regulatory phenotype when cocultured with CD4⁺ T cells [37], while monocytes. Interestingly, while MDSC induce FoxP3⁺ Tregs, CD14⁺HLA-DR⁺ cells induced a different T helper subtype, Th17 cells [40].

We have been able to identify S100A9 as one potential marker, which can be used to distinguish MDSC from monocytes [41]. Unfortunately, S100A9 is expressed intracellularly and cannot be used to isolate cells for functional studies. Genetic comparison of CD14⁺HLA-DR^{neg/low} MDSC and CD14⁺HLA-DR⁺ monocytes revealed a number of differences including higher expression of ATRA related genes in MDSC. ATRA is an important factor for the generation of Tregs [42], which correlates with the induction of T regulatory phenotype by MDSC.

Th17 cells

It has been shown that Th17 cells are associated with poor outcome in HCC [43]. In other tumors, conflicting reports have been published on the role of Th17 cells in tumor immunity [43,44]. We have shown an increase in the frequency of Th17 cells in peripheral blood and Th17 related cytokines (IL-17, IL-23) in tumor supernatants from HCC patients. This observation prompted us to investigate whether Th17 cells have an effect on CD8⁺ T cell function. *In vitro* studies, demonstrated Th17 cells inhibit IFN- γ production and proliferation by CD8⁺ T cells. Further analysis revealed that only the CCR4⁺CCR6⁺ subpopulation of Th17 cells was responsible for this effect, which prompted us to examine CCR4⁺CCR6⁺ Th17 cell populations in HCC patients in more detail. Interestingly, we found only an increase of CCR4⁺CCR6⁺ Th17 cells in peripheral blood from patients with HCC but not of CCR4^{neg}CCR6⁺ Th17 cells [45]. Due to the low frequency of CCR4⁺CCR6⁺ Th17 cells in peripheral blood it was not possible to isolate enough cells for functional studies *in vitro*. However, we were able to examine CCR4⁺CCR6⁺ CD4⁺ T cells from peripheral blood of HCC patients and compare their function with CCR4^{neg}CCR6⁺ CD4⁺ T cells. In order to eliminate any potential effect of Tregs, CCR4⁺CCR6⁺ CD4⁺ T cells and

CCR4^{neg}CCR6⁺ CD4⁺ T cells were isolated from CD25^{low/neg}CD127⁺ PBMC. As shown in Figure 3 CCR4⁺CCR6⁺ CD4⁺ T cells demonstrated a marked suppression of CD8⁺ T cell responses in contrast to CCR4^{neg}CCR6⁺CD4⁺ T cells.

Neutrophils

Neutrophils represent the most abundant leukocytes. Proinflammatory cytokine IL-17 is a critical mediator for the recruitment of neutrophils in the tumor microenvironment. It has been shown that Neutrophils are not only engaged in inflammatory responses, but can also modulate angiogenesis. In HCC, peritumoral infiltration of neutrophils has been described to positively correlate with angiogenesis progression at tumor-invading edge of HCC patients [46].

Monocytes

The role of monocytes in the tumor microenvironment of HCC has been thoroughly studied by Zheng's groups. It has been shown that expression of PD-L1 on the surface of monocytes and macrophages in the peritumoral stroma suppressed T cell responses [47]. In further studies, they also demonstrated that monocytes not only suppressed T cell function directly, but also induced an IL-17 secreting effector CD8⁺ T cell (Tc17 cells) [48] as well as Th17 cells [49].

In summary, HCC has developed multiple cellular and molecular pathways to evade potent anti-tumor immune responses. In this review, we have summarized cellular immune escape mechanisms in HCC. We believe that it will be important in the future to test treatment modalities, which will target this cell population especially in trials which aim to enhance anti-tumor immune responses. Initial studies targeting Tregs using low dose cyclophosphamide and a recently published study evaluating the effect of anti-CTLA4 treatment in HCC [50] do not only provide preliminary evidence that it is possible to augment anti-tumor immunity but also demonstrate that this type of approach is safe and needs further development and evaluation in the near future.

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References

1. Korangy F, Ormandy LA, Bleck JS, Klempnauer J, Wilkens L, Manns MP, et al. Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. *Clin Cancer Res.* Jul.2004 10:4332–4341. [PubMed: 15240519]
2. Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, et al. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. *Hepatology.* Apr.2011 53:1206–1216. [PubMed: 21480325]
3. Behboudi S. Alpha-fetoprotein specific CD4 and CD8 T cell responses in patients with hepatocellular carcinoma. *WJH.* 2010; 2:256. [PubMed: 21161007]
4. Behboudi S, Boswell S, Williams R. Cell-mediated immune responses to α -fetoprotein and other antigens in hepatocellular carcinoma. *Liver International.* Apr.2010 30:521–526. [PubMed: 20040052]
5. Zhang H-G, Chen H-S, Peng J-R, Shang X-Y, Zhang J, Xing Q, et al. Specific CD8⁺ T cell responses to HLA-A2 restricted MAGE-A3 p271–279 peptide in hepatocellular carcinoma patients without vaccination. *Cancer Immunol Immunother.* May 24.2007 56:1945–1954. [PubMed: 17522859]

6. Korangy F, Höchst B, Manns MP, Greten TF. Immune responses in hepatocellular carcinoma. *Dig Dis.* 2010; 28:150–154. [PubMed: 20460904]
7. Korangy F, Höchst B, Manns MP, Greten TF. Immunotherapy of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol.* Jun.2010 4:345–353. [PubMed: 20528121]
8. Greten TF, Manns MP, Korangy F. Immunotherapy of hepatocellular carcinoma. *J Hepatol.* Dec. 2006 45:868–878. [PubMed: 17046096]
9. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet.* Jan.2001 27:18–20. [PubMed: 11137992]
10. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet.* Jan.2001 27:20–21. [PubMed: 11137993]
11. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell.* May 30.2008 133:775–787. [PubMed: 18510923]
12. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. *Journal of Experimental Medicine.* Jul.2002 196:247–253. [PubMed: 12119349]
13. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol.* Jun.2002 2:389–400. [PubMed: 12093005]
14. Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, Boehmer von H, et al. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF- β signals in vivo. *Proc Natl Acad Sci USA.* Jan.2005 102:419–424. [PubMed: 15623559]
15. Boehmer von H. Mechanisms of suppression by suppressor T cells. *Nat Immunol.* Apr.2005 6:338–344. [PubMed: 15785759]
16. Gavin MA, Torgerson TR, Houston E, DeRoos P, Ho WY, Stray-Pedersen A, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc Natl Acad Sci USA.* Apr.2006 103:6659–6664. [PubMed: 16617117]
17. Baron U, Floess S, Wieczorek G, Baumann K, Grützkau A, Dong J, et al. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. *Eur J Immunol.* Sep.2007 37:2378–2389. [PubMed: 17694575]
18. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med.* Sep.2004 10:942–949. [PubMed: 15322536]
19. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res.* Sep.2006 12:5423–5434. [PubMed: 17000676]
20. Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay NEH, et al. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. *Clin Cancer Res.* Jan. 2006 12:465–472. [PubMed: 16428488]
21. Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res.* Mar.2005 65:2457–2464. [PubMed: 15781662]
22. Unitt E, Rushbrook SM, Marshall A, Davies S, Gibbs P, Morris LS, et al. Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. *Hepatology.* Apr. 2005 41:722–730. [PubMed: 15791620]
23. Yang XH, Yamagiwa S, Ichida T, Matsuda Y, Sugahara S, Watanabe H, et al. Increase of CD4+ CD25+ regulatory T-cells in the liver of patients with hepatocellular carcinoma. *J Hepatol.* Aug. 2006 45:254–262. [PubMed: 16600416]
24. Shen X, Li N, Li H, Zhang T, Wang F, Li Q. Increased prevalence of regulatory T cells in the tumor microenvironment and its correlation with TNM stage of hepatocellular carcinoma. *J Cancer Res Clin Oncol.* Mar.2010 136:1745–1754. [PubMed: 20221638]

25. Kobayashi N, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, et al. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res.* Feb. 1;2007 13:902–911. [PubMed: 17289884]
26. Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology.* Jun. 2007 132:2328–2339. [PubMed: 17570208]
27. Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol.* Jun.2007 25:2586–2593. [PubMed: 17577038]
28. Zhou J, Ding T, Pan W, Zhu L-Y, Li L, Zheng L. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. *Int J Cancer.* Oct.2009 125:1640–1648. [PubMed: 19569243]
29. Yang Z-Q, Yang Z-Y, Zhang L-D, Ping-Bie, Wang S-G, Ma K-S, et al. Increased liver-infiltrating CD8+FoxP3+ regulatory T cells are associated with tumor stage in hepatocellular carcinoma patients. *Hum Immunol.* Dec.2010 71:1180–1186. [PubMed: 20870003]
30. Greten TF, Ormandy LA, Fikuart A, Höchst B, Henschen S, Hörning M, et al. Low-dose cyclophosphamide treatment impairs regulatory T cells and unmasks AFP-specific CD4+ T-cell responses in patients with advanced HCC. *J Immunother.* Jan.2010 33:211–218. [PubMed: 20139774]
31. Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol.* Jul.2011 11:802–807. [PubMed: 21237299]
32. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol.* Mar.2012 12:253–268. [PubMed: 22437938]
33. Young MR, Newby M, Wepsic HT. Hematopoiesis and suppressor bone marrow cells in mice bearing large metastatic Lewis lung carcinoma tumors. *Cancer Res.* Jan.1987 47:100–105. [PubMed: 2947676]
34. Zhao F, Obermann S, Wasielewski von R, Haile L, Manns MP, Korangy F, et al. Increase in frequency of myeloid-derived suppressor cells in mice with spontaneous pancreatic carcinoma. *Immunology.* Sep.2009 128:141–149. [PubMed: 19689743]
35. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest.* Oct.2006 116:2777–2790. [PubMed: 17016559]
36. Haile LA, Gamrekelashvili J, Manns MP, Korangy F, Greten TF. CD49d is a new marker for distinct myeloid-derived suppressor cell subpopulations in mice. *J Immunol.* Jul.2010 185:203–210. [PubMed: 20525890]
37. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Krüger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology.* Jul.2008 135:234–243. [PubMed: 18485901]
38. Cai L, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol.* Sep.2008 DOI: 10.1016/j.clim.2008.08.012.
39. Hoechst B, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, Wedemeyer H, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology.* Sep.2009 50:799–807. [PubMed: 19551844]
40. Hoechst B, Gamrekelashvili J, Manns MP, Greten TF, Korangy F. Plasticity of human Th17 cells and iTregs is orchestrated by different subsets of myeloid cells. *Blood.* Jun.2011 117:6532–6541. [PubMed: 21493801]
41. Zhao F, Hoechst B, Duffy A, Gamrekelashvili J, Fioravanti S, Manns MP, et al. S100A9 a new marker for monocytic human myeloid derived suppressor cells. *Immunology.* Feb.2012 DOI: 10.1111/j.1365-2567.2012.03566.x.
42. Wang J, Huizinga TWJ, Toes REM. De novo generation and enhanced suppression of human CD4+CD25+ regulatory T cells by retinoic acid. *The Journal of Immunology.* Sep.2009 183:4119–4126. [PubMed: 19717521]

43. Zhang J-P, Yan J, Xu J, Pang X-H, Chen M-S, Li L, et al. Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients. *J Hepatol.* May 1.2009 50:980–989. [PubMed: 19329213]
44. Zou W, Restifo NP. T(H)17 cells in tumour immunity and immunotherapy. *Nat Rev Immunol.* Apr.2010 10:248–256. [PubMed: 20336152]
45. Zhao F, Hoechst B, Gamrekashvili J, Ormandy L, Voigtländer T, Wedemeyer H, et al. Human CCR4⁺CCR6⁺Th17 cells suppress autologous CD8⁺ T cell responses. *Journal of Immunology.* Apr.2012 in press.
46. Kuang D-M, Zhao Q, Wu Y, Peng C, Wang J, Xu Z, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol.* May 1.2011 54:948–955. [PubMed: 21145847]
47. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *Journal of Experimental Medicine.* Jun.2009 206:1327–1337. [PubMed: 19451266]
48. Kuang D-M, Peng C, Zhao Q, Wu Y, Zhu L-Y, Wang J, et al. Tumor-activated monocytes promote expansion of IL-17-producing CD8⁺ T cells in hepatocellular carcinoma patients. *The Journal of Immunology.* Aug.2010 185:1544–1549. [PubMed: 20581151]
49. Kuang D-M, Peng C, Zhao Q, Wu Y, Chen M-S, Zheng L. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. *Hepatology.* Jan.2010 51:154–164. [PubMed: 19902483]
50. Melero, I.; Sangro, B.; Riezu-Boj, JI.; Iñarrairaegui, M.; Lasarte, JJ.; Sarobe, P., et al. Antiviral and antitumoral effects of the anti-CTLA4 agent tremelimumab in patients with hepatocellular carcinoma (HCC) and chronic hepatitis C virus (HCV) infection: Results from a phase II clinical trial. *Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; Chicago, Illinois Philadelphia (PA).* 2012 Mar 31-Apr 4; AACR; p. 1-1.2012 Abstract nr4387 2012 Apr. 27

Figure 1. Spontaneous NY-ESO specific antibody responses can be found in at least 50% of all HCC, which express NY-ESO

HCC tumor samples were analyzed by RT-PCR for the NY-ESO1. The proportion of NY-ESO-1 positive samples (total number 12) are shown in red. In six cases anti-NY-ESO-1 specific antibody responses were detected in serum from HCC patients, in 3 cases the serum was negative for NY-ESO specific antibodies and in 3 cases no serum was available for analysis.

Figure 2.
Overview of immune cells, which either suppress tumor specific immune responses or, which are suppressed in the tumor environment of HCC

Figure 3. CCR4⁺CCR6⁺ CD4⁺ T cells from patients with HCC suppress proliferation of CD8⁺ T cells

CCR4⁺CCR6⁺ or CCR4^{neg}CCR6⁺ CD4⁺ T cells were isolated from peripheral blood of patients with HCC and g-irradiated. Irradiated cells were cultured with autologous CD3⁺CD8⁺ T cells at different ratios. CD8⁺ T cell proliferation was analyzed. Data shown is the cumulative results of 7 independent experiments. White bars represent proliferation of CD3⁺CD8⁺ T cells alone. *p<0.05.