The liver, liver metastasis and liver cancer: a special case for immunotherapy with cytokines and immunostimulatory monoclonal antibodies

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The liver as a secondary lymphoid organ

The liver is placed between the gut and systemic circulation. One of its many functions is to defend the organism from major portal entry of bacteria and viruses and to avoid the entry and free circulation of endotoxins [1]. Specialized liver macrophages, Kupffer cells, lining in the lumen of the peculiar liver capillaries, termed sinusoids, account for the most part of these functions. For reasons not entirely understood, the liver contains a high density of NK cells in comparison with other organs. NK T cells with invariant T-cell receptors are also very abundant in this organ under healthy conditions [2,3]. Such large granular lymphocytes are termed 'pit cells' at this location [4]. The liver also contains APCs of different kinds, including conventional dendritic cells (DCs) and plasmacytoid DCs. Importantly for tumor immunology, CD8α DCs specialized in priming and tolerizing CD8 T cells are present in the liver [5]. Lymph drainage from the liver is canalized by a virtual space between the hepatocytes and the fenestrated capillaries (Disse's space) and drains along portal tracts toward lymph nodes at the liver hilum.

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The immunologic outcome of liver transplantations tells us that this organ is somehow specialized as a tolerogenic organ under steady state conditions. Its functions are presumably more related to immunological silence than to immunological response. It is, for example, important to consider that rejection of kidney allografts is less intense and frequent when the liver is concomitantly transplanted. Although not completely clarified, this is attributed to the fact that liver APCs create bone marrow microchimerism and tolerize against histocompatibility antigens [6]. Therefore, successful liver engraftment requires less immunosuppression than other organs [7]. Nonetheless, immune responses can be primed in the liver. As Nicholas Crispe points out, "The liver's immune system is held in a baseline state of active tolerance, which can be reversed by sufficiently strong pathogen-specific signals" [1]. Local IL-10 production by macrophages and continuous desensitization of microbial sensing receptors are believed to play a major role in the immunosuppressive environment of the healthy liver. However, this can be overruled in many pathological conditions that provide the most conspicuous examples of chronic inflammatory infiltrates. In this regard, NK and NK T cells probably play a crucial early role in mounting responses to microbial insults when sensing alarm signals denoting microbial presence or stressful cell death.

It should be kept in mind that the normal immune behavior of the liver is profoundly disrupted in cirrhosis and chronic hepatic inflammation. Blood circulation and lymph drainage change, and the subpopulations of immune system cells completely differ to what is observed at steady state. To understand malignancy and the liver we should realize that: primary liver cancer most often originates from cirrhotic liver, while a liver metastasis typically nests in a healthy organ. In the first case the tumor is benefiting from inflammatory factors that drive transformation and cancer promotion [8], while in the second case it is benefiting from the local immunosuppressive mechanisms that secure immune escape.

Future Medicine part of fsg

IL-12 in the treatment of primary liver cancer & colorectal cancer metastasis

Increasing evidence suggests that immune responses are involved in the control of cancer and that the immune system can be manipulated in different ways to recognize and fight against cancer cells [9]. However, in the liver, the presence of chronic HCV or HBV infection may limit the success of immunotherapy in patients with hepatocellular carcinoma (HCC) because these viruses have been found to be able to negatively modulate the host immune response. Although there is a limited clinical experience regarding the use of immunotherapy in liver cancer contrary to more classical immunogenic tumors, an important amount of information regarding the use of IL-12 for liver cancer therapy has been accumulated in recent years.

> *"IL-12 is a multifunctional cytokine, perhaps one of the most potent, which displays anti-tumor activity in a number of experimental models."*

IL-12 is a multifunctional cytokine, perhaps one of the most potent, which displays anti-tumor activity in a number of experimental models. It can induce activation of NK cells, cytotoxic T lymphocytes and the induction of a Th1 type of response [10,11]. It also inhibits tumor angiogenesis and enhances the expression of adhesion molecules on vascular endothelium [11], acting mainly via downstream IFN-γ. Unfortunately, IL-12 was demonstrated to be unacceptably toxic in humans when systemically administered as a recombinant protein; however, using gene therapy strategies it has been possible to induce efficacious immunity with limited toxicity [12]. Therefore, gene therapy approaches significantly increased cytokine expression in the liver without excessive systemic levels of the cytokine [13]. In Phase I clinical studies carried out in advanced gastrointestinal patients, including HCC, short-term IL-12 expression using first generation recombinant adenoviruses demonstrated the proof-of-concept that tumoral/peritumoral production of IL-12 stimulated tumor infiltration by effector immune cells, followed by an objective tumor response in a minority of patients [14]. More recently, advances in the application of long-term expression vectors for IL-12 delivery or the use of conditioned replicative viruses expressing IL-12 seems to provide new clues for the therapeutic benefit of IL-12 [15]. We have observed several limitations for the use of IL-12-based gene therapy protocols. For example, biologically active IL-12 can transiently reduce the activity of its promoter, particularly inhibiting the function of drug-inducible systems in nonintegrative DNA vectors, an effect probably mediated by IFN-γ [15]. Another therapeutic limitation associated with the use of IL-12-based gene therapy was the activation of immunosuppressive mechanisms such as the induction of $Foxp3^+$ regulatory T cells and immunosuppressive molecules (e.g., PD-1, PD-L1, VEGF, CTLA-4, IDO) [16].

An emerging role of IL-15 for the treatment of tumors nesting in the liver?

IL-15 is a cytokine that increases CD8 and NK cells, both in numbers and activity [17]. A Phase I clinical trial using recombinant IL-15 includes an escalation dose of rhIL15 in patients with melanoma, renal and colon cancer (NCT01021059) and preliminary results from the first-in-human clinical trial suggest that bolus infusion showed unexpected signs of acute toxicity at low doses, including severe hypotension and high fever; continuous infusion may be a way to overcome this problem.

IL-15 is a cytokine that physiologically acts as a co-stimulatory molecule. Experimental evidence supports that instead of being a soluble factor this cytokine acts tethered to the IL-15Rα chain on one cell triggering the signal translation machinery of IL-2Rβγ on a juxtaposed cell. This is termed transpresentation of IL-15 [18]. We have recently used gene transfer to the liver of the cytokine complexed to a soluble transpresenting IL-15Rα form. The strategy renders the local proliferation and activation of CD8 and NK lymphocytes with effects against syngeneic mouse colon cancer (MC38) grafted to the liver [Ochoa MC *et al.* Immunotherapeutic and toxic effects of a triple fusion protein encompassing apolipoprotein A-I, interleukin-15 and the interleukin-15 receptor-α sushi domain (2012), Submitted]. Fusion protein strategies to improve the pharmacokinetics of this cytokine are in progress and are being tested in models of liver cancer.

Immunostimulatory monoclonal antibodies

The ability to manipulate at will the immune response against cancer would be absolutely thrilling. A step forward is to engineer agonist and antagonist molecular tools that would act on receptors of cells of the immune system or on the cytokines that they use to communicate with each other. Fully human monoclonal antibodies

(mAbs) are ideally suited to perform these pharmacological agonist or antagonist functions both from a pharmacokinetic and pharmacodynamic point of view [19]. Two major kinds of immunostimulatory mAb are under clinical development: blockers of inhibitory receptors and agonists of costimulatory receptors [19,20]. Of the first kind, anti-CTLA-4 and anti-PD-1 mAbs are the most advanced in clinical development. Ipilimumab has been approved for the treatment of metastatic melanoma [21,22] and antibodies directed to PD-1 and its ligand PD-L1 have demonstrated extremely promising clinical activity for melanoma, renal cell carcinoma and lung cancer [23,24]. Liver tumors are not exception and are also amenable to treatment by these agents. There are plenty of reported cases with liver metastasis of melanoma showing evidence for complete and partial responses to ipilimumab [25] and to tremelimumab (a second anti-CTLA-4 mAb under development) [26]. These cases make the proof-of-concept that the liver is not a sanctuary for these immune-potentiating treatments.

> *"There are plenty of reported cases with liver metastasis of melanoma showing evidence for complete and partial responses to ipilimumab ... and to tremelimumab..."*

In the case of tremelimumab, a clinical trial has been conducted in patients with HCC and hepatitis C showing three partial responses out of 17 evaluated patients according to RECIST criteria and important decreases in HCV viremia [Sangro B *et al.* A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C (2012), Submitted].

In the case of the anti-PD-1 mAb, a clinical trial for HCC Phase I is planned. Again, there is abundant evidence for clinical activity against liver metastasis of melanoma, colorectal carcinoma and renal cell carcinoma [27]. A major quest for predictive biomarkers for the response is ongoing, but it is important to know that surface expression of B7-H1 (PD-L1) on malignant cells seems to be a major although not the only predictor. A Phase I trial whose results have not been reported yet for patients with hepatitis C infection with an anti-PD-L1 mAb has been completed (NCT00703469) and this same antibody offers signs of clinical benefit against metastatic tumors in a multiple dose Phase I trial [24].

The main drawback of these new classes of agent is that they can cause inflammatory complications either as autoimmune conditions or inappropriate responses to the flora in the skin or the gut. Serious adverse events of this kind have been recorded. Ulcerative colitis outstands in the case of CTLA-4 blockade and severe pneumonitis in a small fraction of patients exposed to PD-1 blockade [23]. In the case of ipilimumab, combined with dacarbazine a significant fraction of the patients showed signs of hepatitis whose nature is not well defined [22].

Other antibodies behave as agonists of costimulatory receptors of the TNF receptor family. These include CD40, CD137 (4-1BB), CD134 (OX40), GITR and CD27. The first three have undergone clinical trials with signs of clinical activity [27,28]. CD40 and CD137 have been shown to elicit liver inflammation in mice with polyclonal T lymphocyte liver infiltrates. In clinical trials with anti-CD137 this has been a serious obstacle limiting doses [27]. However, in the case of liver tumors this could be considered a blessing or a curse because it may help to focus the response on the liver harboring malignancy. Unfortunately, this selective activity has not been observed in rodent models yet [29]. Liver tumors of metastatic or primary origin promise to be a principal battle field in the development of these promising sets of therapeutic antibodies, which act as activation tools of the immune response against cancer.

Combinatorial immunotherapy & liver cancer

The effectiveness of the immunotherapeutic approaches described above is deeply hampered by the tumor's hostile repertoire that suppresses the effector immune response [30]. Seeking combinatorial treatment approaches, involving more than one agent, that would be synergistic against cancer, it was possible to increase the efficacy of immunotherapy by combining different procedures, such as chemotherapy, transarterial embolization, anti-angiogenic molecules, immunostimulatory mAbs, DC vaccination and other immunotherapy approaches. Some of these combinations demonstrated a synergistic rather than an additive effect in terms of antitumoral response. For example, elimination or inhibition of Treg and/or myeloid-derived supressor cells activity by low-dose cyclophosphamide (single-dose or metronomic schedule), gemcitabine or 5-fluorouracil may modify tumor immunosuppressive microenvironment, thereby increasing the efficacy of immunotherapy [31–33]. Although the mechanisms of action of chemotherapeutic agents are not fully understood, it has been recently demonstrated that autophagy is

necessary to generate an immunogenic cell death and an optimal anti-tumoral effect [34]. It was recently reported in an orthotopic HCC model in mice that sunitinib, which failed to induce anti-tumoral response as monotherapy in the clinic, combined with adoptive transfer of tumor antigen-specific $CD8^+$ T cells led to elimination of established tumors, a response associated with suppression of STAT3 and a block in T-cell tolerance [35]. In another model established in mice using H22 cells, Ma *et al.* demonstrated the synergistic activity of the combination between B7H3- and vasostatin-expressing plasmids after intratumoral injection [36]. Anti-tumoral response was associated with enhanced infiltration of NK cells, activated $CD8^+$ T cells and inhibited tumor angiogenesis. Huang *et al.* assessed a combined anti-angiogenic therapy with immunotherapy using adenoviruses encoding PEDF, endostatin and cytokines (GM-CSF and IL-12), in a woodchucks with HCC; suffering from a large and multifocal model that closely replicates the human condition [37]. Vectors were administered via the hepatic artery and resulted in a potent anti-tumoral effect by inducing a significant $CD3^+$ T and NK cell infiltration.

Conclusion & future perspective

If immunotherapy of cancer were a car we should do the following: releasing the brakes (tamper with inhibitory receptors of the immune system) and pressing the accelerators (locally provided cytokines or immunostimulatory mAbs). To ignite the engine by priming against tumor antigens will be also needed and this can be achieved by making a tumor look like a vaccine or with active vaccination for identified tumor antigens. To make a tumor look like a vaccine, local destruction of tumor cells under proinflammatory conditions seems appropriate.

In mouse models, Smyth *et al.* have pioneered combinations of new targeted immunotherapies with excellent results using various immunostimulatory mAbs used in conjunction [38], findings that have been also reported by other investigators [39], including liver metastasis examples. Testing early in development of combinatorial strategies poses regulatory, legal and business obstacles [40]. However, such 'artificial' hurdles need to be overcome because excellent efficacy is predicted both by preclinical models and early clinical trials. Malignant diseases arising from the liver or nesting this organ should be no exception.

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References

1 Crispe IN. The liver as a lymphoid organ. *Annu. Rev. Immunol.* 27, 147–163 (2009).

2 Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. *Hepatology* 47(2), 729–736 (2008).

3 Swain MG. Natural killer T cells within the liver: conductors of the hepatic immune orchestra. *Dig. Dis.* 28(1), 7–13 (2010).

4 Wisse E, Luo D, Vermijlen D, Kanellopoulou C, De Zanger R, Braet F. On the function of pit cells, the liver-specific natural killer cells. *Semin. Liver Dis.* 17(4), 265–286 (1997).

5 O'Connell PJ, Morelli AE, Logar AJ, Thomson AW. Phenotypic and functional characterization of mouse hepatic CD8 α^+ lymphoid-related dendritic cells. *J. Immunol.* 165(2), 795–803 (2000).

6 Starzl TE, Demetris AJ, Trucco M *et al.* Systemic chimerism in human female recipients of male livers. *Lancet* 340(8824), 876–877 (1992).

7 Bishop GA, Ierino FL, Sharland AF *et al.* Approaching the promise of operational tolerance in clinical transplantation. *Transplantation* 91(10), 1065–1074 (2011).

8 Stauffer JK, Scarzello AJ, Jiang Q, Wiltrout RH. Chronic inflammation, immune escape and oncogenesis in the liver: a unique neighborhood for novel intersections. *Hepatology* doi:10.1002/hep.25674 (2012) (Epub ahead of print).

9 Matar P, Alaniz L, Rozados V *et al.* Immunotherapy for liver tumors: present status and future prospects. *J. Biomed. Sci.* 16, 30 (2009).

10 Mazzolini G, Prieto J, Melero I. Gene therapy of cancer with interleukin-12. *Curr. Pharm. Des.* 9(24), 1981–1991 (2003).

11 Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev.* 13(2), 155–168 (2002).

12 Leonard JP, Sherman ML, Fisher GL *et al.* Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-γ production. *Blood* 90(7), 2541–2548 (1997).

13 Berraondo P, Prieto J, Gonzalez-Aseguinolaza G. Advances in interleukin-12 gene therapy for acquired liver diseases. *Curr. Gene Ther.* 9(2), 62–71 (2009).

14 Sangro B, Mazzolini G, Ruiz J *et al.* Phase I trial of intratumoral injection of an adenovirus encoding interleukin-12 for advanced digestive tumors. *J. Clin. Oncol.* 22(8), 1389–1397 (2004).

15 Reboredo M, Zabala M, Mauleon I *et al.* Interleukin-12 inhibits liver-specific drug-inducible systems *in vivo*. *Gene Ther.* 15(4), 277–288 (2008).

16 Zabala M, Lasarte JJ, Perret C *et al.* Induction of immunosuppressive molecules and regulatory T cells counteracts the antitumor effect of interleukin-12-based gene therapy in a transgenic mouse model of liver cancer. *J. Hepatol.* 47(6), 807–815 (2007).

17 Waldmann TA, Lugli E, Roederer M *et al.* Safety (toxicity), pharmacokinetics, immunogenicity, and impact on elements of the normal immune system of recombinant

human IL-15 in rhesus macaques. *Blood* 117(18), 4787–4795 (2011).

18 Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and its therapeutic implications in cancer. *Trends Pharmacol. Sci.* 33(1), 35–41 (2012).

19 Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat. Rev. Cancer* 7(2), 95–106 (2007).

20 Peggs KS, Quezada SA, Allison JP. Cancer immunotherapy: co-stimulatory agonists and co-inhibitory antagonists. *Clin. Exp. Immunol.* 157(1), 9–19 (2009).

21 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).

22 Robert C, Thomas L, Bondarenko I *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* 364(26), 2517–2526 (2011).

23 Topalian SL, Hodi FS, Brahmer JR *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366(26), 2443–2454 (2012).

24 Brahmer JR, Tykodi SS, Chow LQ *et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* 366(26), 2455–2465 (2012).

25 Saenger YM, Wolchok JD. The heterogeneity of the kinetics of response to ipilimumab in metastatic melanoma: patient cases. *Cancer Immun.* 8, 1 (2008).

26 Ribas A. Clinical development of the anti-CTLA-4 antibody tremelimumab. *Semin. Oncol.* 37(5), 450–454 (2010).

27 Ascierto PA, Simeone E, Sznol M, Fu YX, Melero I. Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies. *Semin. Oncol.* 37(5), 508–516 (2010).

28 Beatty GL, Chiorean EG, Fishman MP *et al.* CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331(6024), 1612–1616 (2011).

29 Dubrot J, Milheiro F, Alfaro C *et al.* Treatment with anti-CD137 mAbs causes intense accumulations of liver T cells without selective antitumor immunotherapeutic effects in this organ. *Cancer Immunol. Immunother.* 59(8), 1223–1233 (2010).

30 Mazzolini G, Murillo O, Atorrasagasti C *et al.* Immunotherapy and immunoescape in colorectal cancer. *World J. Gastroenterol.* 13(44), 5822–5831 (2007).

31 Malvicini M, Ingolotti M, Piccioni F *et al.* Reversal of gastrointestinal carcinoma-induced immunosuppression and induction of antitumoural immunity by a combination of cyclophosphamide and gene transfer of IL-12. *Mol. Oncol.* 5(3), 242–255 (2011).

32 Ko HJ, Kim YJ, Kim YS *et al.* A combination of chemoimmunotherapies can efficiently break self-tolerance and induce antitumor immunity in a tolerogenic murine tumor model. *Cancer Res.* 67(15), 7477–7486 (2007).

33 Vincent J, Mignot G, Chalmin F *et al.* 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res.* 70(8), 3052–3061 (2010).

34 Michaud M, Martins I, Sukkurwala AQ *et al.* Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* 334(6062), 1573–1577 (2011).

35 Avella DM, Li G, Schell TD *et al.* Regression of established hepatocellular carcinoma is induced by chemoimmunotherapy in an orthotopic murine model. *Hepatology* 55(1), 141–152 (2012).

36 Ma L, Luo L, Qiao H *et al.* Complete eradication of hepatocellular carcinomas by combined vasostatin gene therapy and B7H3-mediated immunotherapy. *J. Hepatol.* 46(1), 98–106 (2007).

37 Huang KW, Wu HL, Lin HL *et al.* Combining antiangiogenic therapy with immunotherapy exerts better therapeutical effects on large tumors in a woodchuck hepatoma model. *Proc. Natl Acad. Sci. USA* 107(33), 14769–14774 (2010).

38 Uno T, Takeda K, Kojima Y *et al.* Eradication of established tumors in mice by a combination antibody-based therapy. *Nat. Med.* 12(6), 693–698 (2006).

39 Melero I, Martinez-Forero I, Dubrot J, Suarez N, Palazon A, Chen L. Palettes of vaccines and immunostimulatory monoclonal antibodies for combination. *Clin. Cancer Res.* 15(5), 1507–1509 (2009).

40 Martinez-Forero I, Okada H, Topalian SL, Gajewski TF, Korman AJ, Melero I. Workshop on immunotherapy combinations society for immunotherapy of cancer annual meeting Bethesda, November 3, 2011. *J. Transl. Med.* 10(1), 108 (2012).