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Combinatorial immunotherapy strategies for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common liver malignancy. The prognosis for HCC patients greatly varies according to the stage at diagnosis. Overall it is poor, with a 5-year survival rate of approximately 5–6%. Immunotherapeutic interventions represent a novel and effective therapeutic tool. However, only few immunotherapy trials for HCC have been conducted so far with contrasting results, suggesting that significant improvements are needed. Indeed, the liver is characterized by a strong intrinsic immune suppressive microenvironment which needs to be counterbalanced with immune stimulatory approaches. Therefore, the implementation of combinatorial protocols combining immune stimulatory strategies with specific immunotherapy approaches could result in a dramatic improvement of efficacy and clinical outcome in HCC patients.

The present review aims at describing the state of the art in immunotherapy strategies for HCC and future perspectives.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with both viral and non-viral origin, and accounts for about 6% of all new cancer cases diagnosed worldwide (nearly 750 000 new cases/year). It is the third and the fifth leading cause of death from cancer globally in men and women, respectively. The age-standardized incidence rate (ASR) per 100 000 per year of HCC greatly varies in different regions. It is about 9.5 in Southern Europe and Northern America but 31.9 and 22.2 in Eastern and South-Eastern Asia, respectively (http://globocan.iarc.fr/).

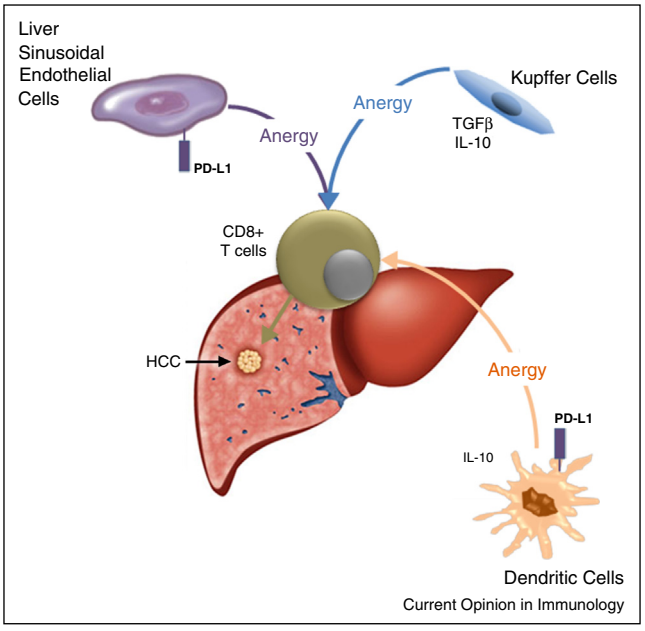
The overall prognosis for HCC patients is poor, with a dismal 5-year survival rate of approximately 5–6% [1,2]. A range of therapies are used in the management of HCC according to the extent and severity of liver disease. Indeed, the majority of patients are diagnosed when disease precludes surgical approaches and can be treated only with loco-regional therapies with limited survival benefits. Moreover, tumor recurrence is observed in 50–80% of patients at 5 years after treatment, with most relapses occurring within 2 years [3•]. Therapeutic options in advanced unresectable HCC are limited to Sorafenib which is the only approved therapy confirmed to provide a limited increase in survival of 2.3–2.8 months [4,5,6,7].

In this framework, immunotherapeutic interventions, including cancer vaccines, may represent a novel and effective therapeutic tool for HCC. Only few clinical trials have been conducted so far, showing that patients can be successfully vaccinated and tumor antigen-specific T cells can be expanded (reviewed in [8]). However, clinical responses have been disappointing. Indeed, the liver is characterized by a strong intrinsic immune suppressive microenvironment which may represent a major impediment to an effective anti-tumor activity elicited by a therapeutic cancer vaccine [9•]. Several cells are involved in inducing such intra-hepatic tolerogenicity, including hepatocytes which have been shown to induce an anergic phenotype in $CD8^+$ T cells [10,11,12]. Furthermore, three distinct subsets of phagocytic cells have been identified to play a role as 'tolerogenic' antigen presenting cells (APCs): liver sinusoidal endothelial cells (LSECs), Kupffer cells and liver dendritic cells (DCs) (reviewed in [9•]).

It is beyond a doubt that such an inherent immune-suppressive tumor environment needs to be taken into account and counterbalanced when immunotherapeutic approaches are designed and implemented in HCC, or else they will likely result in poor outcomes (Figure 1).

The present review article takes into account the different immunotherapies and the immune stimulatory

Figure 1



Cells of the HCC microenvironment. The three major subsets of 'tolerogenic' antigen presenting cells (APCs) are shown: liver sinusoidal endothelial cells (LSECs), Kupffer cells and liver dendritic cells (DCs). They all contribute to induce an anergic phenotype in $CD8^+$ T cells.

approaches, as well as their combination available and evaluated in HCC.

Immunotherapy approaches for HCC

A limited number of immunotherapy trials for HCC have been conducted based on several strategies, with yet modest results. They are based on cellular and non-cellular strategies, which may be further divided into active and passive immunotherapy strategies.

Cellular immunotherapy

Different solid tumors, including HCC, refractory to conventional therapies show promising responsiveness to adoptive immunotherapy treatments. Here we briefly describe passive as well as active cellular immunotherapy strategies for HCC, taking into consideration only those which have been evaluated in clinical trials and showing promising outcomes.

Passive immunotherapy strategies

Cytokine-induced killer cells

Cytokine-induced killer (CIK) cells are characterized by expression of both the CD3 T cell biomarker and the CD56 NK cell biomarker and can be generated from human peripheral blood mononuclear cells (PBMC) treated with IFN-γ, anti-CD3 antibody and IL-2 [13].

CIK cells show strong activity against a broad spectrum of targeted solid and non-solid tumors, including HCC, with minimal toxicity and no graft-*vs*-host disease [14,15]. In particular, immunotherapy based on CIK cells induces a significant increase in both overall survival (OS) and progression-free survival (PFS) of HCC patients. This has been shown with or without association with other therapeutic modalities as assessed by two recent meta-analysis [16,17•]. However, the efficacy of CIK immunotherapy for HCC needs to be further validated, by increasing the number of evaluated HCC patients.

NK and NKT cells

NK cells belong to the innate immune system and play a critical role in the host defense against solid tumors. Such an effect is exerted by direct killing of tumor cells as well as by releasing immunomodulatory cytokines which can activate cytotoxic leukocytes (reviewed in [18]).

Anti-tumor activity of NK cells is significantly inhibited in patients with advanced HCC [19]. Such a functional impairment may be induced by myeloid-derived suppressor cells (MDSCs) [20] and has been associated also with increased Tregs [21]. Reactivation of NK cells has been shown to be effective on HCC in preclinical studies [22], suggesting that HCC patients could benefit from enhancement of NK cells functionality [23]. However, the number of clinical studies performed to now are not sufficient to corroborate the efficacy of NK cell immunotherapy in HCC. Indeed, a study demonstrated that blood circulating NK cells can be activated in HCC patients by radiofrequency ablation (RFA) [24]. Moreover, two ongoing clinical trials are designed to assess NK cell therapy combined with liver resection (NCT02008929) or liver transplantation for HCC (NCT01147380).

NK T (NKT) cells are a heterogeneous group of T cells playing a role in regulating the anti-tumor immunity [25]. They can exert both immunosuppressive and immunoactivation functions [26]. A phase I clinical trial using autologous NKT cells to treat advanced HCC is now ongoing with no results available yet (NCT01801852).

Adoptive cell transfer of Tumor-infiltrating lymphocytes.

Adoptive cell therapy (ACT) is based on the infusion of autologous T cells activated and expanded *ex vivo* [27]. In particular, tumor-infiltrating lymphocytes (TILs) are mostly T cells found in tumor and their density is directly correlated with improved clinical outcomes [28,29]. Such findings suggest that indeed the tumor is immunogenic and that the host is able to mount an immune response to cancer cells. However, it is not sufficient to contain and/or eradicate the tumor mass.

Autologous TILs derived from tumor patients were originally generated and expanded *ex vivo* for subsequent re-infusion in melanoma patients [30]. ACT has been