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Challenges in cancer vaccine development for hepatocellular carcinoma

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Summary

Hepatocellular carcinoma (HCC) is the most common liver malignancy, representing the third and fifth leading cause of death from cancer worldwide in men and women, respectively.
The main risk factor for the development of HCC is the hepatitis B and C virus (HBV and HCV) infection; non-viral causes (e.g., alcoholism and aflatoxin) are additional risk factors.
HCC prognosis is generally poor because of the low effectiveness of available treatments and the overall 5-year survival rate is approximately 5–6%.
In this framework, immunotherapeutic interventions, including cancer vaccines, may represent a novel and effective therapeutic tool. However, only few immunotherapy trials for HCC have been conducted so far with contrasting results, suggesting that improvements in several aspects of the immunotherapy approaches need to be implemented.
In particular, identification of novel specific tumor antigens and evaluation of most advanced combinatorial strategies could result in unprecedented clinical outcomes with great beneficial effect for HCC patients.
The state of the art in immunotherapy strategies for HCC and future perspectives are reported in the present review.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and accounts for about 6% of all new cancer cases diagnosed worldwide (nearly 750,000 new cases/year). It is the third and fifth leading cause of death from cancer globally in men and women, respectively. The age-standardized incidence rate (ASR) of HCC in men in Europe, adjusted to the European Standard Population, is about 8 per 100,000, with a peak in Southern Europe of 10.5 per 100,000 (http://globocan.iarc.fr/). The overall prognosis for HCC patients is poor, with a dismal 5-year survival rate of approximately 5–6% [1].

The role of immune microenvironment in hepatocarcinogenesis

The liver shows an inherent tolerogenicity, to prevent an aberrant immune response to gut-derived microbial products which are constantly re-circulated through the liver. Several cells are involved in inducing such intra-hepatic tolerogenicity, including hepatocytes which have been shown to prime naïve T cells in the absence of co-stimulation, T cells which ultimately acquire an anergic cytotoxic phenotype undergoing a clonal deletion [2,3]. 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). LSECs express the inhibitory molecule B7-H1/PD-L1, which induces antigen-specific CD8+ T-cell tolerance interacting with PD-1 on the T cells [4,5]. Moreover, LSECs negatively regulates hepatic T-cell immune response [6] inducing CD4+ T-cell tolerance and death [7]. Kupffer cells exert their tolerogenic activity by producing the anti-inflammatory molecules transforming growth factor beta (TGF-β), IL-10, prostaglandin E2 (PGE2), as well as expressing the inhibitory molecule B7-H1 and eliminating high affinity antigen-specific CD8+ T cells that enter the liver [8–10]. Liver-resident DCs show an IL-10-secreting phenotype [11–13], inducing Th2 polarization of CD4+ T cells [14], regulatory T-cell (Treg) induction and poor antigen recall responses [15,16].

Furthermore, hepatic stellate cells (HSCs) have been proposed to contribute to immune tolerance by induction of apoptosis of activated T cells in mice [17,18].

The inherent intra-hepatic immunosuppressive environment is further exacerbated by the chronic inflammation status induced by chronic hepatitis [19–21]. In addition, several HCV proteins are able to directly alter cytokine expression and modulate the tumor microenvironment, contributing to HCC development (reviewed elsewhere [22,23]).

Once HCC is established, the tumor microenvironment is characterized by a leukocyte infiltrate, whose main cellular components include tumor-associated macrophages (TAMs) and T cells. Such microenvironment highly supports TAM polarization towards activated M2-macrophages that express high levels of

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cytokines such as IL-10 and TGF-β, which, in turn, support Treg recruitment as well as development of T helper 17 polarized immune response [24].

The incidence of tumor infiltrating lymphocytes (TILs) in HCC is significantly low, confirming the presence of an intrahepatic suppressive mechanism. In particular, HCC prognosis is strictly related to the predominant TILs' population identified in the tumor [25]. Indeed, high levels of Treg cells play a central role in promoting tumor growth and progression, being associated with a poor HCC prognosis [26].

In summary, the intrahepatic tolerogenic and immune suppressive microenvironment appears to be a very favorable milieu for a multi-step process potentially leading to HCC development (Fig. 1), when the risk factor conditions (e.g., chronic hepatitis infection) occur. Such inherent immunological uniqueness needs to be taken into high account when immunotherapeutic approaches are designed and implemented, or they will likely result in poor outcomes.

Loco-regional treatments for HCC with immunological implication

When applicable, surgery (i.e., tumor resection and liver transplantation) represents the standard treatment of HCC, since a 5-year survival rate is achieved in 70% of treated patients [27–29].

However, several loco-regional non-surgical treatments (i.e., radiofrequency (RF), thermal and non-thermal ablation, transarterial chemoembolization (TACE)) provide a second line of therapy for patients with unresectable HCC or for those who are not eligible for liver transplantation, with an extremely variable 3 to 5-year survival rates according to the disease stage at the time of treatment [30].

The spontaneous regression of untreated tumors has been consistently reported in different tumor diseases after thermoablation of distant tumor masses, suggesting a tumor-specific immune activation induced by the loco-regional treatment [31–34]. This effect is likely the result of the release of cellular material by necrotic cells, generated by ablation, which is able to induce a local inflammation and, ultimately, a specific immune response [35–37]. Such an effect of providing tumor antigens to the immune system is further boosted by the recruitment and activation of immune effector cells at the tumor site, including DCs, which results in the induction of effector as well as memory immune response [38,39]. Moreover, it has been shown that the removal of tumor tissue leads to depletion of Treg reverting the intratumoral balance towards effective antitumor immunity [40]. Other loco-regional treatments may exert similar positive effects on the anti-tumor immune response (for a most complete review, see [41]).

Nevertheless, such immune responses induced by the loco-regional treatments are not sufficient to induce a full tumor protection but show a great potential as adjuvanting strategy to improve immunogenicity of specific immunotherapy approaches [42]. Such adjuvanting effect, however, needs to be more extensively demonstrated in randomized clinical trials.

Immunotherapy approaches for HCC

A limited number of immunotherapy trials for HCC have been conducted based on several strategies, with yet modest results. Cytokines have been used to activate subsets of immune cells and/or increase the tumor immunogenicity [43,44]. Further strategies have been based on infusion of tumor infiltrating lymphocytes or activated peripheral blood lymphocytes [45–47]. Alternatively, direct delivery of genetically modified or designer T cells (dTc) into the hepatic artery has been recently proposed as a promising novel strategy and is currently evaluated in a phase I human clinical trial (ClinicalTrials.gov Identifier: NCT01373047). Indeed, the latter strategy has recently been successfully used for treatment of different cancers and several human clinical trials are currently ongoing (reviewed in [48]).

Alternatively, considering active immunotherapy strategies (i.e., therapeutic vaccination), the number of human clinical trials published to date is extremely small. The first HCC vaccine clinical trial was conducted by Butterfield *et al.* based on CD8+ T-cell epitopes specific for alpha fetoprotein (AFP), showing the generation of AFP-specific T-cell responses in vaccinated subjects [49]. To improve the immune response, the same authors performed a subsequent phase I/II trial administering AFP epitopes presented by autologous DCs loaded *ex vivo*. This treatment, however, resulted only in transient CD8+ T-cell responses, possibly caused by the lack of CD4+ help [50,51]. To overcome this limitation and to increase the number of tumor associated antigens (TAAs) targeted by the immune response elicited by the vaccine, few vaccine approaches, based on autologous DCs pulsed *ex vivo* with a lysate of the autologous tumor [52] or of hepatoblastoma cell line HepG2 [53,54], have been evaluated in human clinical trials, showing limited improvements in clinical outcomes.

The last clinical trial in the literature is based on a combination of low-dose cyclophosphamide treatment followed by a telomerase peptide (GV1001) vaccination which did not show antitumor efficacy [55].

Few phase I/II clinical trials, testing immunotherapy strategies for the treatment of HCC, are currently ongoing in recruiting participants (Table 1).

Specific limiting factors need to be addressed in order to improve the limited outcomes from the clinical trials, some of which are discussed below.

Limiting factors in immunotherapy approaches for HCC

HCC-specific tumor associated antigens (TAAs)

HCC-specific TAAs are limited in number and can be classified in distinct classes, including (a) *widely occurring, overexpressed antigens* (i.e., telomerase reverse transcriptase – TERT; Wilms' tumor 1-WT-1) [56–59]; (b) *oncofetal antigens* (i.e., alpha fetoprotein – AFP, glypican 3 – GPC3) [60–62]; and (c) *cancer/testis (CT) antigens* (i.e., MAGE-A, SSX-2, NY-ESO-1) [63]. Only HLA class-I restricted epitopes from TAAs of the first two classes (i.e., TERT and AFP) have been tested in human clinical trials with limited results [49,55].

New and more specific TAAs and/or epitopes should be identified, both HLA class I and II restricted, aiming at inducing CD4+ as well as CD8+ T-cell activation. Indeed, clonal expansion and acquisition of cytolytic functions of CD8+ CTL are obtained only in the presence of the helper function provided by CD4+ T helper (Th) cells [64–67].

To this aim, a systems biology approach could be applied, integrating multiple high-throughput "omics" technologies (reviewed in [68]). Novel TAAs identified by these high-throughput technologies are currently analyzed by immune-informatics

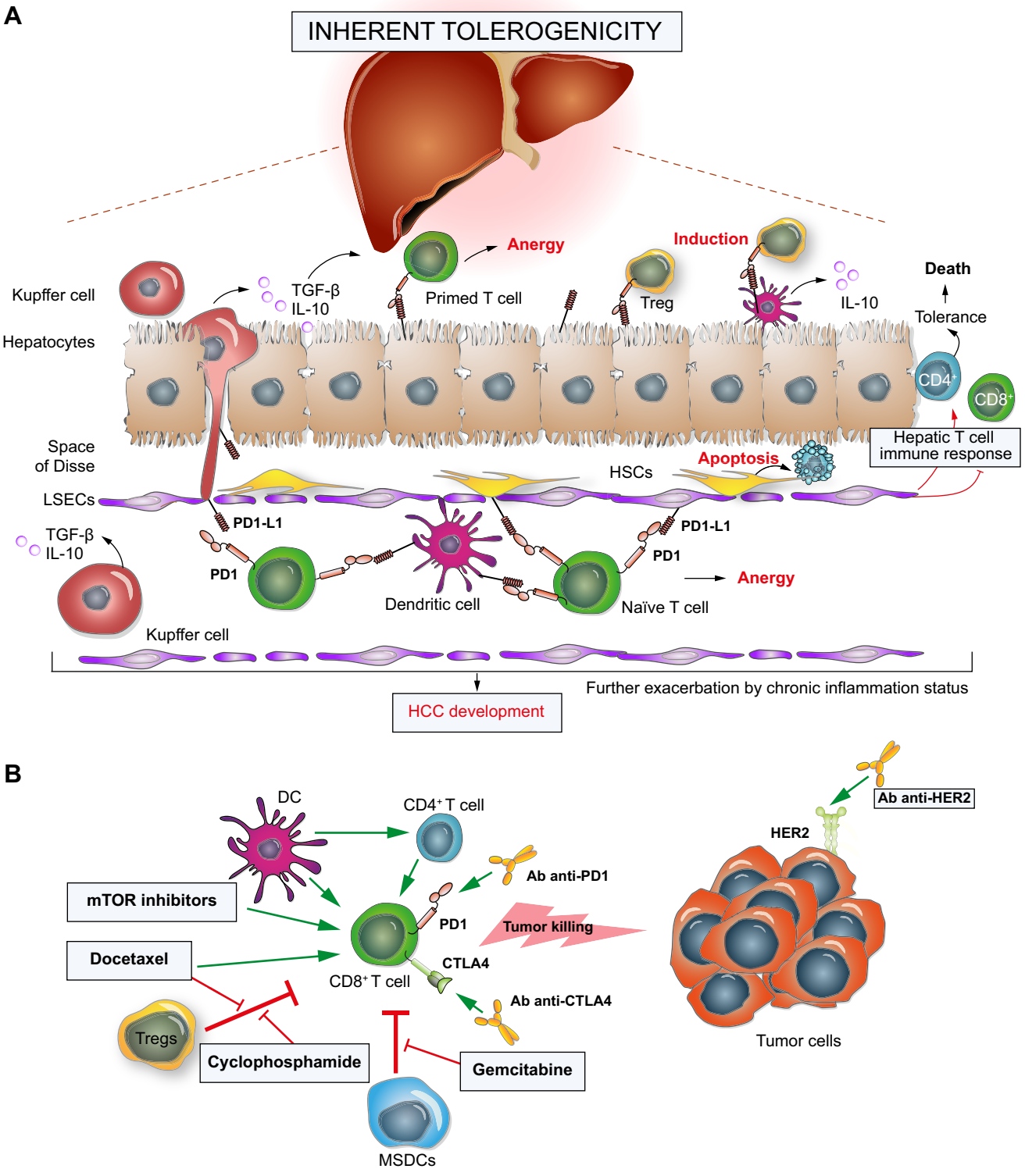

Fig. 1. Liver tumor microenvironment and counterbalancing strategies. (A) Representation of cell populations in the liver microenvironment with promoting and inhibiting function on tumor cell killing activity of CD8 + T cells. (B) Combinatorial strategies to counterbalance the inhibitory activities and potentiate the immune response to cancer vaccine are indicated in boxed text.

Table 1. Published and ongoing HCC cancer vaccines evaluated in human clinical trials.

Antigen	Vaccine strategy	Ref.
AFP	Peptides	[57]
AFP	DC pulsed	[58,59]
Autologous tumor lysate	DC pulsed	[60]
Tumor cell line lysate	DC pulsed	[61,62]
Telomerase	Peptides (GV1001)	[63]
NY-ESO-1	Peptide	Clinical trial identifier: NCT01522820

algorithms to predict specific epitopes that interact with MHC complexes in order to stimulate a T-cell-associated immune response [69–73]. Prediction algorithms, however, cannot take into considerations all the biological variables related to the complexity of the process governing the peptide fragmentation by the proteasome and the transportation to HLA class I molecules in the endoplasmic reticulum, via the transporter associated with antigen processing (TAP). To overcome such limitations, alternative strategies based on high resolution mass spectrometry (MS) have been recently used for directly sequencing peptides presented by HLA molecules (HLA ligandome) from tumor cells, to identify naturally processed class I and class II tumor-associated peptides [74]. This strategy, indeed, allows the identification of T-cell epitopes presented by the tumor cells, thus representing a valid target of T cells, and it has been employed to identify the HLA ligandome for glioblastoma (GB) [75], renal cell cancer (RCC), and colorectal cancer (CRC). Cancer vaccines based on peptides identified with this strategy have been developed. A safety phase I clinical trial is currently ongoing for the GB; for RCC and CRC, cancer vaccines phase II clinical trials have been conducted, both showing the association of T-cell responses with clinical benefit [76,77]. A phase III efficacy trial is currently ongoing for the RCC cancer vaccine.

Combinatorial strategies

Improvement of the immune response elicited by active cancer immunotherapies may be achieved also by improving the immunogenicity of the vaccine antigen and/or counterbalancing the immune-suppressive tumor environment. To this aim, several lines of evidence suggest that combination of immunotherapy and cancer standard-of-care therapies (i.e., chemotherapy) may provide better results than individual treatments (reviewed in [78,79]).

Cytotoxic chemotherapies induce an *immunogenic cell death* with the release of danger signals from tumor cells, which can promote anti-tumor immunity, polarizing DCs towards a pro-inflammatory phenotype which drives a T helper 1 (Th1) response (reviewed in [80,78]). Moreover, cyclophosphamide is toxic to immunosuppressive Treg cells and a metronomic regimen has been shown to improve anti-tumor cell response [81,82] as well as cancer vaccine efficacy [83–87]. Similarly, gemcitabine selectively kills myeloid-derived suppressor cells (MDSCs) *in vitro* and *in vivo* [88], and has been tested in combination with cancer vaccines [89–91]. Docetaxel has been reported to modulate different cell subsets, enhancing CD8+ function and deleting Tregs [92] and has been evaluated in several human clinical trials to test the enhancement of immune response to cancer vaccine [93,94].

Targeted cancer therapies may induce remarkable tumor regression in cancer patients positive for the target pathway/protein, but the relatively rapid selection of tumor cells resistant to such therapies represents a significant limitation to their utility [95]. Nevertheless, as for the cytotoxic chemotherapies, several observations indicate that targeted therapies may help improve anti-tumor immune responses elicited by immunotherapies (reviewed in [79]). In particular, the combination of cancer vaccines and immune checkpoint blockade to prevent T-cell anergy may result in a potentiated anti-tumor immune response (reviewed in [96]). Alternatively, targeted therapies may improve antigen presentation (e.g., anti-HER2 Abs) [97,98] or maintain the activation of vaccine-specific T cells and promote their differentiation into memory T cells (e.g., mTOR inhibitors) [99].

Specifically concerning the HCC, combinatorial strategies have been evaluated in a single clinical trial based on a combination of low-dose cyclophosphamide treatment combined with a telomerase peptide (GV1001) vaccination [55]. A phase I clinical trial is currently ongoing to evaluate a combination therapy based on rapamycin and NY-ESO-1 fusion protein vaccine, in patients with cancers expressing the NY-ESO-1 antigen, including HCC

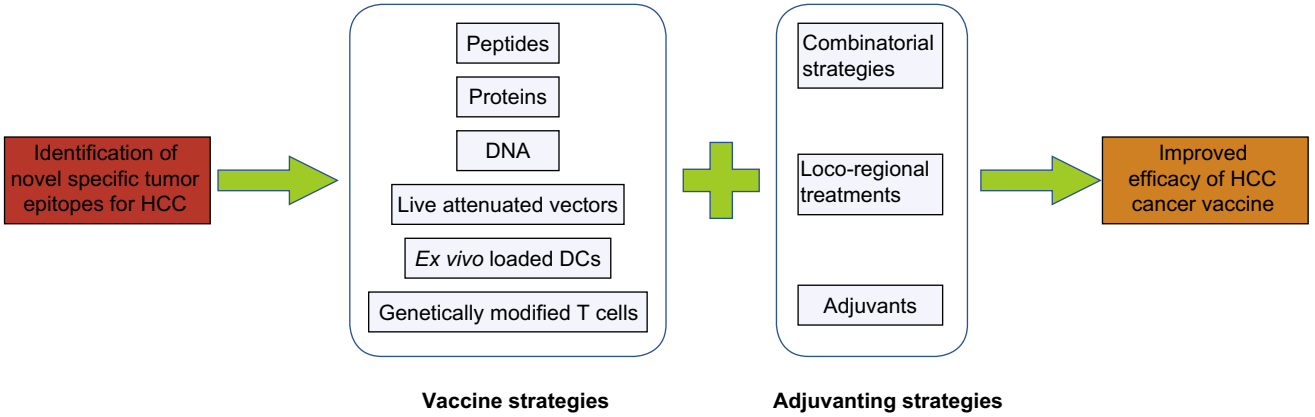

Fig. 2. Proposed scheme for improving HCC cancer vaccines. The identification of novel specific tumor epitopes for HCC will represent the ground for development of new vaccine concepts, based on any of the listed strategies or combination of them. This will take great advantage from the immune potentiating activities of any of the listed adjuvanting strategies or combination of them. The final expected outcome is the improved efficacy of cancer vaccine.

(ClinicalTrials.gov Identifier: NCT01522820). Considering all the data generated for other cancer models, it is reasonable to predict that HCC immunotherapies may take significant advantages by designing combinatorial protocols including chemotherapeutic agents to improve the experienced limited clinical outcomes (Fig. 1B).

Key Points

- Current treatment options for hepatocellular carcinoma have been summarized
- The intrinsic tolerogenic environment of the liver organ has been discussed
- The limited outcome of the immunotherapy approaches for HCC has been discussed
- Possible solutions have been proposed, in particular, discovery of new tumor associated antigens (TAAs) and evaluation of combinatorial strategies

Concluding remarks

Treatment of HCC is a primary goal, given its poor prognosis for the lack of an effective therapy. The liver is intrinsically an immune-suppressive environment, further worsened by chronic hepatitis infection, which represents a favorable context for cancer development. Each of the current available treatments is palliative and immunotherapy has been only partially explored with limited clinical outcomes. Improving the knowledge on molecular and antigenic characteristics of HCC, to identify more specific and immunogenic tumor-associated antigens, and testing the potential benefits of the combinatorial strategies, to increase the vaccine immunogenicity and efficiently counterbalance the immune-suppressive environment, will very likely result in unprecedented clinical outcomes with great beneficial effects for HCC patients (Fig. 2).

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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