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New vaccination strategies in liver cancer

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1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer globally. The age-standardized incidence rate (ASR) of HCC in men in Europe, adjusted to the European Standard Population, is about 8 per 100,000 (http://globocan.iarc.fr/). Hepatitis B and C virus (HBV and HCV) chronic infection is the main risk factor for the development of HCC [1,2].

Therapies for HCC are dependent on the stage of disease with an extremely variable 5-year survival rate [3,4]. Surgery represents the standard treatment for HCC in the early stages with a 5-year survival rate of 70% among treated patients [5–7]. Non-surgical loco-regional therapies are implemented for HCC patients showing a more advanced disease, with highly variable 3 to 5-year survival rates [8] and tumor recurrence in more than 50% of patients at 5 years after treatment [9]. Sorafenib is the only approved systemic therapy in advanced unresectable HCC, providing a very limited survival benefit [10–13]. Very recently, the RESORCE trial (Regorafenib After Sorafenib in Patients With Hepatocellular Carcinoma) has shown that regorafenib can be used as second-line treatment in patients with advanced stage HCC who were progressing while on sorafenib. Although the study showed only a modest 2.8-month improvement in overall survival, with a 38% reduction in the risk of death, this was the first trial to show any benefit of a second-line treatment [14].

In such a scenario, immunotherapy strategies for HCC may represent a key therapeutic tool to improve clinical outcome in HCC patients. In particular, therapeutic cancer vaccines could be very promising. This is a still open field of research, given that only very few cancer vaccine trials for HCC have been conducted so far with yet modest results [15–17].

1.1. Vaccine approaches based on whole tumor lysate

Tumor lysates are considered the optimal strategy to elicit a broad anti-tumor immune response provided that all potential tumor-associated antigens (TAAs) are effectively presented to the T cells, without selecting only specific TAAs that may not include the most relevant ones. Moreover, the immunogenicity of TAAs delivered by tumor lysates is potentiated by cell-derived adjuvanting molecules and damage-associated molecular patterns (DAMPs) which are strong activators of the innate immunity. The strongest limitation of such a vaccine approach is the low representation of such relevant TAAs among the vast predominance of cellular self-antigens, which may result in unproductive elicitation of anti-tumor immune responses [18].

Very few early stage clinical trials have been conducted in advanced stage HCC patients using whole tumor cell lysates (Fig. 1). The first trial using autologous DCs loaded with autologous tumor lysate was conducted by Lee et al. [19]. The vaccination was shown to be safe and 21/31 patients (67.7%) exhibited signs of efficacy (i.e. 4 partial responses and 17 stable diseases). Moreover, the boosted schedule resulted in an improved 1-year survival rate ($63.3 \pm 12.0\%$ vs. $10.7 \pm 9.4\%$; $p < 0.001$). A second trial using autologous DCs pulsed *ex vivo* with a liver tumor cell line lysate (HepG2) was reported by Palmer et al. [20]. Also in this case, the vaccination was safe and 7/25 patients (28%) showed signs of efficacy. More recently El Ansary et al. reported an additional trial

Abbreviations: HCC, hepatocellular carcinoma; ASR, age-standardized rate; HBV, hepatitis B virus; HCV, hepatitisC virus; TAAs, Tumor-associated antigens; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; AFP, α-fetoprotein; MAGE-1, Melanoma-associated antigen 1; IFNγ, interferon gamma; GPC3, Glypican-3; CTL, cytotoxic T lymphocytes; OS, overall survival; MRP3, Multidrug resistance-associated protein 3; CT, cancer testis; SSX-1, synovial sarcoma X; hTERT, human telomerase reverse transcriptase; GM-CSF, granulocyte-macrophage colony-stimulating factor; TTP, time to progression; TTSP, time to symptomatic progression; GB, glioblastoma; RCC, renal cell cancer; CRC, colorectal cancer; Tregs, T regulatory cells.

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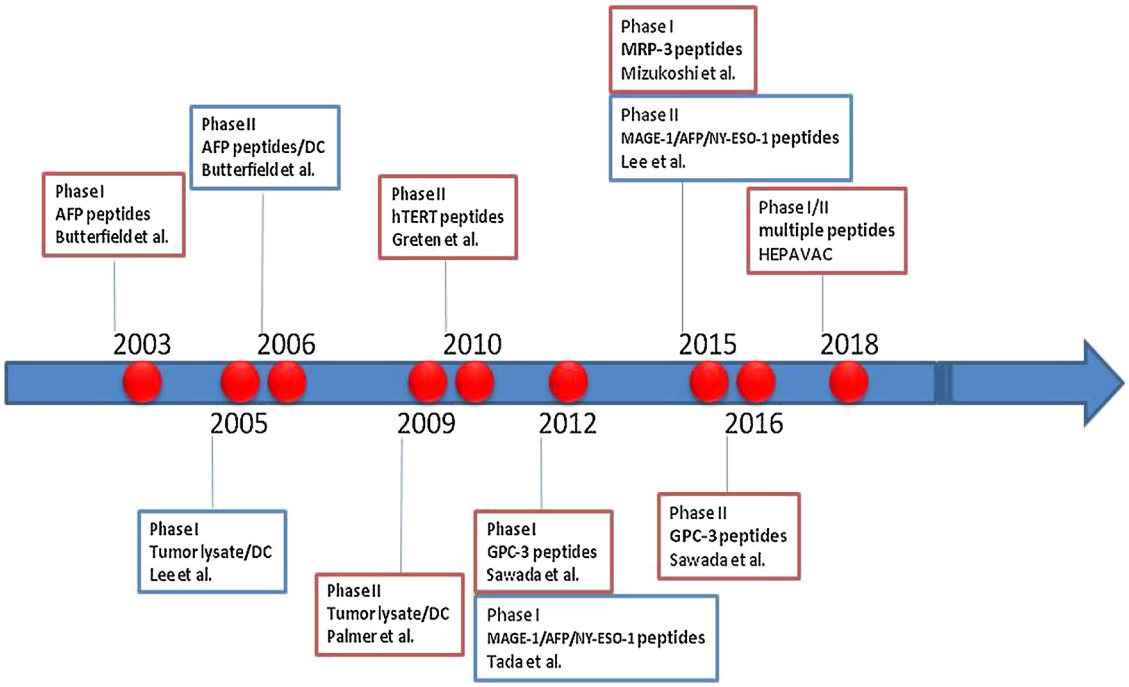

Fig. 1. Timeline of HCC cancer vaccine development. List of different cancer vaccine approaches and antigens evaluated in HCC patients in clinical trials during the years.

to evaluate a similar HepG2-pulsed DC vaccine [21]. 11/15 patients (73.3%) showed either partial response or stable disease, although the median survival time was only 4 months.

1.2. Vaccine approaches based on specific antigens

Cancer vaccines are mostly based on peptides representing only a single TAA and frequently the induction of antigen-specific T cells does not correlate with significant clinical outcome [22]. The vast majority of such vaccines include wild type shared antigens which are expressed at low levels also in some benign tissues. This leads to central and peripheral tolerance mechanisms with selection of T cells with low-affinity T cell receptors (TCR) or to severe autoimmune toxicities when targeted with high affinity engineered TCRs [23,24]. Alternatively, tumor-specific neoantigens derive from non-synonymous somatic mutations resulting in mutated peptides, which may be presented on the cell surface and recognized by T cells. Because such mutated peptides are specific to tumor tissues only, neoantigen-specific T cells are not subject to central tolerance and should not induce autoimmune adverse effects. As a result, neoantigens appear to represent ideal targets for T cell-based cancer immunotherapy [25].

Concerning HCC, only a few early phase clinical trials have been conducted in the setting of advanced stage HCC, targeting single over-expressed TAAs. Hitherto, such TAAs were all shared wild type antigens.

1.3. A-Fetoprotein (AFP)

Alpha fetoprotein (AFP) is an oncofetal antigen over-expressed in most HCCs and was the first target antigen selected for HCC-specific immunotherapy approaches. The first pilot phase I clinical trial showed the immunogenicity of AFP peptides in HCC patients, regardless of the high circulating levels of AFP [26]. A subsequent clinical trial was conducted to evaluate autologous DCs pulsed with the same AFP peptides in AFP-positive HCC patients. Results showed both an expansion of AFP-specific T cells as well as increased IFNγ-producing AFP-specific T cell responses to at least one of the peptides included in the mix [27].

1.4. Glypican-3 (GPC3)

Glypican-3 (GPC3) is overexpressed in about 80% of HCCs and correlated with a poor prognosis [28–30]. A vaccine based on two GPC3 peptides was evaluated in advanced stage HCC patients in a nonrandomized, open-label, phase I clinical trial. A specific CTL response was observed in most of the vaccinated subjects and the CTL frequency nicely correlated with OS. Indeed, the median OS was 12.2 months in patients with GPC3-specfic CTL frequencies >50, compared with 8.5 months in those with GPC3-specfic CTL frequencies <50 ($p = 0.033$) [31]. A following phase II clinical trial showed that the same vaccine combined with surgery resulted in a lower tumor recurrence rate compared to surgery alone (28.6% vs. 54.3% and 39.4% vs. 54.5% at 1 and 2 y, respectively; $p = 0.346$, 0.983). Moreover, the GPC3 expression on tumors strongly correlated with the recurrence rate at 1 year [32].

1.5. Multidrug resistance-associated protein 3 (MRP3)

Multidrug resistance-associated protein 3 (MRP3) is highly expressed in HCC tissue and MRP3-specific cytotoxic T cells (CTLs) have the ability to kill HCC cells overexpressing MRP3 [33]. Adding to these observations, safety and immunogenicity of a MRP3-derived peptide has been evaluated in a phase I clinical trial. A MRP3-specific immunity was elicited in 67% of the vaccinated patients. One patient showed a partial response, nine showed a stable disease, and two showed disease progression. The median overall survival time was 14.0 months which is only two months longer than in previous studies [34].

1.6. Cancer-testis antigens

Cancer-testis (CT) antigens are TAAs specifically expressed on tumor tissue and testis [35]. NY-ESO-1 has been detected in about