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FOCUSSED RESEARCH REVIEW

Developments in cancer vaccines for hepatocellular carcinoma

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Abstract Hepatocellular carcinoma (HCC) accounts for about 6 % of all new cancers diagnosed worldwide and represents one of the leading causes of cancer-related death globally in men and women, respectively. The overall prognosis for HCC patients is poor, especially in the majority of patients with more advanced stage of disease. Indeed, in such cases immunotherapeutic strategies may represent a novel and effective tool. A few immunotherapy trials conducted for HCC have provided divergent results, urging the scientific community to explore additional paths to improve efficacy of immunotherapeutic approaches. The "Cancer Vaccine development for Hepatocellular Carcinoma"—HEPAVAC Consortium has been funded by the EU within the FP7 with the goal of developing a novel therapeutic peptide-based cancer vaccine strategy for HCC including both "off-the-shelf" and personalized antigens. This will be one of the very few vaccine trials for HCC and the first multi-epitope, multi-target and multi-HLA allele therapeutic cancer vaccine for such a frequent and aggressive disease with a hitherto high unmet medical need. Feasibility, safety and biological efficacy will be evaluated in a randomized, controlled European multicenter phase I/II clinical trial.

Keywords Hepatocellular carcinoma · Cancer vaccine · Tumor-associated epitopes · HEPAVAC · HLA ligandome · NIBIT 2014

Abbreviations

AFP	Alpha fetoprotein
APVAC	Actively personalized vaccine
ASR	Age-standardized
CIITA	Class II (major histocompatibility complex) transactivator
CRC	Colorectal cancer
DCs	Dendritic cells
dTc	Designer T cells
EGF	Epidermal growth factor
EU	European Union
FoR	Frequency of recurrence
FP7	Framework Programme 7
GB	Glioblastoma
GM-CSF	Granulocyte–monocyte colony-stimulating factor
CPC3	Glypican 3
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HEPAVAC	Cancer Vaccine development for Hepatocellular Carcinoma
IFNγ	Interferon gamma
IGFR	Insulin-like growth factor receptor
IL-12	Interleukin-12
LSECs	Liver sinusoidal endothelial cells

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The members of HEPAVAC Consortium are listed in the "Appendix" section.

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MoA	Mode of action
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
Pts	Patients
QoL	Quality of life
Ras MAPK	Mitogen-activated protein kinases
RCC	Renal cell cancer
RFA	Radiofrequency ablation
RFS	Recurrence-free survival
SD	Stable disease
TAAs	Tumor-associated antigens
TACE	Transcatheter chemoembolization
TAEs	Tumor-associated epitopes
TILs	Tumor infiltrating lymphocytes
TtFR	Time to first recurrence
TUMAPs	Tumor-associated peptides
VEGF	Vascular growth factor

HCC epidemiology

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with both viral and nonviral origin, accounting 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 cancer-related death globally in men and women, respectively. The age-standardized incidence rate (ASR) per 100,000 men per year for HCC greatly varies in different regions. It is about 9.5 in Southern Europe and Northern America but increases to 31.9 and 22.2 in Eastern and Southeastern Asia, respectively (http://globocan.iarc.fr/).

There is a growing incidence of HCC worldwide mostly due to long-lasting chronic HBV and HCV infections acquired in the last century, although incidence and mortality rates are greatly heterogeneous [1–4].

The most frequent risk factors for HCC include chronic viral hepatitis (types B and C), alcohol intake and aflatoxin exposure. However, even though their geographical distribution is uneven, more than 50 % of HCC cases can be attributed to HBV infection, more than 30 % can be attributed to HCV infection, and approximately 15 % can be associated with other causes.

Treatment options and prognosis

The overall prognosis for HCC patients is poor, with a dismal 5-year survival rate of approximately 5–6 % [5, 6]. Indeed, the number of medical interventions tested in HCC is significantly lower compared to other cancers with a high prevalence/incidence worldwide (e.g., lung, breast, colorectal cancers). Therefore, a limited range of therapies are available to be used in the management of HCC according to the extent and severity of liver disease.

Surgery (i.e., liver resection and transplantation) represents the first choice of treatment for HCC in patients with early tumors on an intention-to-treat perspective, achieving a survival of 60–80 % at 5 years [7, 8]. However, 70 % of patients undergoing liver resection show tumor recurrence within 5 years characterized by either intrahepatic metastases or appearance of *de novo* tumor lesions [9]. Several adjuvant treatments to prevent recurrence have been evaluated, but none of these has provided a clear body of evidence for efficacy [10].

However, the majority of patients are diagnosed when disease is not treatable by surgical strategies anymore and can be approached only with loco-regional therapies which include a large panel of choices [11]. Local ablation is the first option for HCC patients at early stages, and radiofrequency ablation (RFA) provides up to a 40–70 % survival rate at 5 years [12]. Indeed, RFA has been considered as a possible alternative to surgical resection in HCC patients with single small lesions, but contrasting clinical outcomes have been reported [13, 14]. Transcatheter chemoembolization (TACE) is the first option for the treatment of intermediate stage and unresectable HCC [15]. Partial response is observed in almost 50 % of patients treated with TACE showing a delayed tumor progression, although survival benefits have not been fully established [16].

Finally, systemic therapeutic options in advanced unresectable HCC are limited to sorafenib which is the only approved therapy confirmed to provide a limited increase of 2.3–2.8 months in survival [17–20]. Different studies have addressed the HCC pathogenesis in order to identify possible additional targets for systemic therapies, suggesting that multiple concurrent molecular mechanisms or pathways are involved (e.g., vascular growth factor (VEGF) signaling; epidermal growth factor (EGF) signaling; Ras MAPK signaling; insulin-like growth factor receptor (IGFR) signaling) [21, 22]. Such studies strongly support the idea that, indeed, combination strategies or targeted therapies are needed to possibly improve clinical outcomes [23].

Immunotherapy approaches for HCC

The limited number of therapeutic options for advanced-stage HCC with effective clinical outcome urges the scientific community to develop new therapeutic tools. In particular, immunotherapy and cancer vaccines may provide

Table 1 Immunotherapy and cancer vaccine approaches for HCC

Strategy	Treatment	No. of enrolled pts.	Finding	Refs.
Immunotherapy	GM-CSF + IFN-γ	15	OS at 26 weeks 40 % OS at 52 weeks 20 %	[25]
	IL-12	9	SD 29 %	[26]
	111In-TIL	3	PR 66 %	[28]
	Activated autologous lymphocytes	150	FoR 59 % versus 77 % TtFR 48 % versus 33 % at 3 y; 38 versus 22 % at 5 y RFS 65 % versus 58 % at 5 y	[29]
Cancer Vaccines	AFP peptides	6	PD 6/6—OS 9.3 mo	[30]
	AFP—DC pulsed	10	PD 9/10—OS 10.4 mo	[31]
	Autologous tumor lysate—DC pulsed	31	PR 4/31—OS 18 mo SD 17/31—OS 13 mo PD 10/31—2.8 mo	[33]
	tumor cell line lysate—DC pulsed	35	PR 1/35—OS N/A SD 6/35—OS N/A PD 18/35—OS N/A N/A 14/35—OS N/A	[34]
	GPC3 peptides	33	PR 1/33—OS 12 mo SD 19/33—OS 13.4 mo PD 13/33—OS 7.4 mo	[36]
	Telomerase peptide	40	SD 17/40—OS 11 months PD 20/40 N/A 3/40	[38]

111In-TIL indium-111-labeled tumor-infiltrating lymphocytes, *AFP* alpha fetoprotein, *DC* dendritic cells, *FoR* frequency of recurrence, *GM-CSF* granulocyte–monocyte colony-stimulating factor, *GPC3* glypican 3, *IFN-γ* interferon gamma, *IL-12* interleukin-12, *mo* months, *N/A* not available, *OS* overall survival, *PD* progressive disease, *PR* partial response, *pts* patients, *RFS* recurrence-free survival, *SD* stable disease, *TtFR* time to first recurrence, *y* years

a significant benefit over current treatment options either in advanced stages or in the adjuvant setting. However, a few immunotherapy trials conducted to date for HCC have provided only modest results (reviewed in [24]). Cytokines have been used to boost anti-tumor immune responses or increase the tumor immunogenicity [25, 26]. Alternatively, tumor-infiltrating lymphocytes (TILs) or activated peripheral blood lymphocytes have been used for intratumoral infusion [27–29].

Similarly, the number of therapeutic clinical cancer vaccine trials performed for HCC is extremely small and results are not satisfactory. T cell epitopes specific for alpha fetoprotein (AFP), used alone or loaded on autologous DCs, have been shown to elicit a specific and transient CD8 + T cell response [30–32]. Alternatively, only limited improvements in clinical outcomes have been observed in HCC patients using autologous DCs pulsed ex vivo with a lysate either of the autologous tumor [33] or of the hepatoblastoma cell line HepG2 [34, 35]. A clinical trial based on peptide vaccine targeting glypican 3 (GPC3) has shown a positive correlation between GPC3-specific CTL frequencies and the median overall survival [36, 37]. Finally, a combination of low-dose cyclophosphamide with a telomerase peptide (GV1001) vaccination did not show any anti-tumor efficacy [38] (Table 1).

Improving immunotherapy strategies for HCC

The limited efficacy of cancer vaccine in HCC may be ascribed to different possible causes, one of these being the strong intrinsic hepatic immunosuppressive microenvironment induced by several residing cell subsets, including hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer cells and liver dendritic cells (DCs) [39–41]. Consequently, combinatorial strategies are needed to address and counterbalance such an immunosuppressive environment in order to improve clinical outcome of cancer vaccine protocols.

An additional cause of the observed limited efficacy is the restricted number of known HCC-specific tumor-associated antigens (TAAs) to be used for eliciting an effective immune response (reviewed in [41]).

New and specific tumor-associated antigens (TAAs) and/or tumor-associated epitopes (TAEs) can be identified by integration of multiple high-throughput "omics" technologies (reviewed in [42]) and validated by immunoinformatics algorithms [43–47]. In this path, the ultimate frontier is represented by the analysis of naturally processed tumor-associated epitopes (so-called HLA ligandome) [48], which has allowed us to identify new epitopes for different tumors

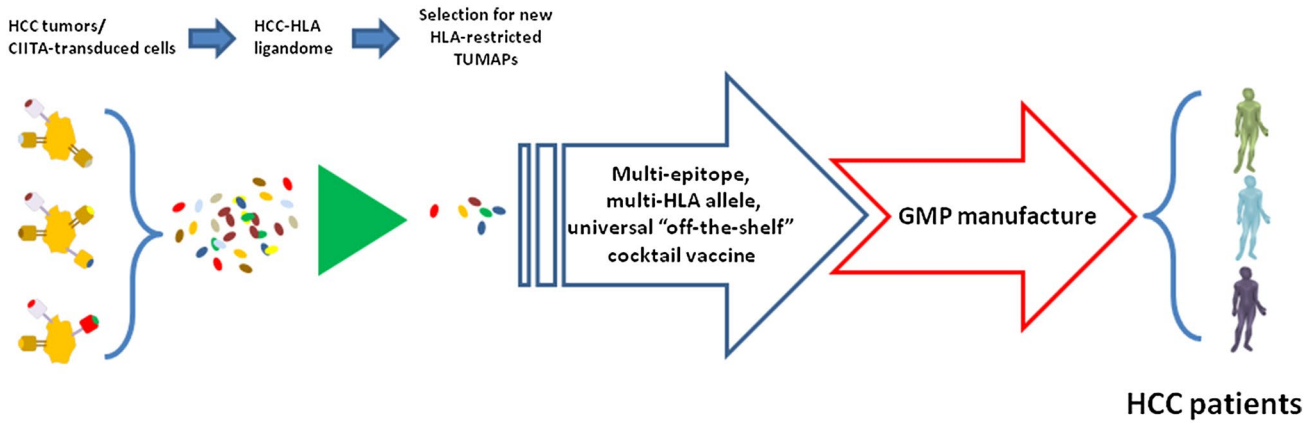

Fig. 1 Strategy and technical process for development of a multi-target, multi-epitope and multi-HLA allele vaccine for hepatocellular carcinoma. HCC tumors as well as CIITA-transduced cells will be analyzed by combined proteomics and genomics strategies for expression of tumor-specific TUMAPs. Shared peptides will be identified, validated immunologically and selected (up to 40) for combination in the HCC universal "off-the-shelf" vaccine

(e.g., glioblastoma—GB [49], renal cell cancer—RCC as well as colorectal cancer—CRC) employed in cancer vaccines phase I/II human clinical trials [50, 51].

Cancer Vaccine development for Hepatocellular Carcinoma: HEPAVAC

Along such a path of multiple high-throughput "omics" technologies applied to cancer vaccinology, the collaborative project "*Cancer Vaccine development for Hepatocellular Carcinoma—HEPAVAC*" has been funded by the EU within the FP7. The main goal of HEPAVAC is to develop a novel therapeutic cancer vaccine strategy for HCC in order to address factors which are believed to have hampered the efficacy of previously tested cancer vaccines (www.hepavac.eu).

In particular, HEPAVAC will generate an "off-the-shelf" vaccine including a cocktail of newly identified HLA class I- and class II-restricted tumor-associated peptides (TUMAPs) among those naturally presented on the membrane of either primary HCC tumor cells or of CIITA-transduced hepatoma cell lines (Fig. 1). The HCC HLA ligandome will be discovered by the XPRESIDENT™ technology (the "Tuebingen approach" [48, 52]), and tumor-specific peptides will be selected according to their broad expression in HCC tumors and low-to-no expression in normal tissues. After immunological validation, such "off-the-shelf" vaccine will be ready to use for any eligible HCC patient characterized by that specific HLA allele background. The vaccination protocol will be complemented, in a subset of vaccinees, by an actively personalized vaccine (APVAC) approach. The latter will include patient-specific naturally processed and presented peptides

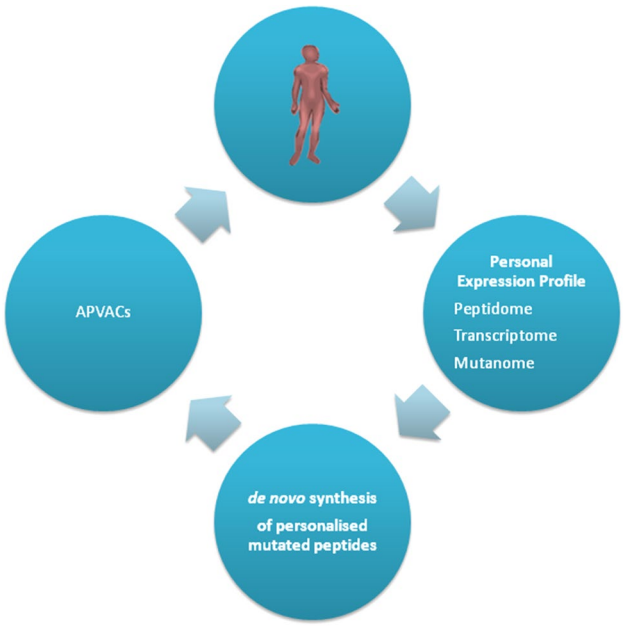

Fig. 2 Strategy of APVAC generation. Each HCC tumors will be analyzed straight after surgery for expression of tumor-specific HLA ligands, and patient-specific peptides will be selected and synthesized de novo. Each patient will then receive a personalized vaccine (APVAC) adapted to his/her own tumor

selected according to integration of genomics, transcriptomics and HLA ligandomics analyses (Fig. 2).

Both the "off-the-shelf" and the personalized vaccine will be combined with a novel and potent RNA-based immunomodulator (RNAdjuvant®) which is based on a noncoding, long-chain RNA molecule able to induce balanced, long-lasting immune responses resulting in a strong anti-tumor activity [53].

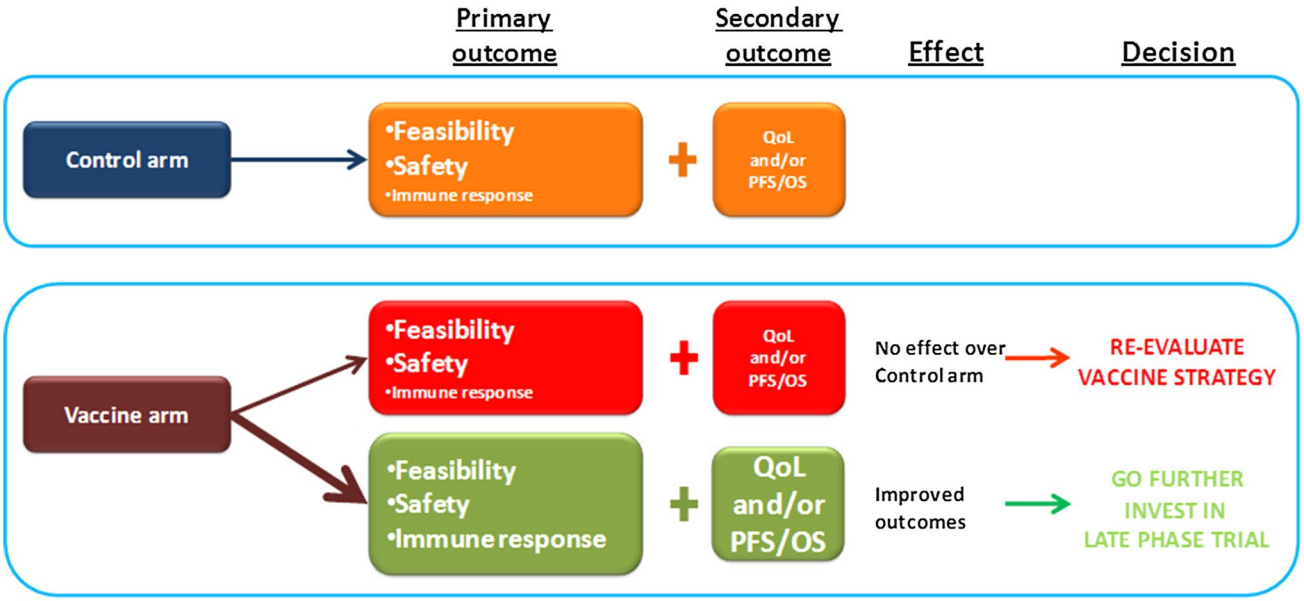

Fig. 3 Scheme of the expected results in the HEPAVAC clinical trial and resulting decisions for further development

Furthermore, to describe the mechanism of action (MoA) and indicate a proof of concept, the project will include a comprehensive T cell immunomonitoring and biomarker program enabling the development of new tools to monitor and predict the clinical outcome of patients.

The HEPAVAC vaccine will be finally evaluated in a randomized controlled European multicenter phase I/II clinical trial to assess feasibility, safety and biological efficacy (Fig. 3).

This will be one of the very few vaccine trials for HCC and the first multi-epitope, multi-target and multi-HLA allele therapeutic cancer vaccine for such a frequent and aggressive disease.

Concluding remarks

HCC is an aggressive disease with a high unmet medical need. In advanced stages, each of the currently available treatments is palliative and immunotherapy has been only partially explored with hitherto limited clinical outcomes. The novel cancer vaccine strategy currently developed by the FP7 EU-funded HEPAVAC Consortium (Grant Agreement No. 602893) will provide results extremely relevant to the cancer vaccine field and hopefully warrant unprecedented clinical outcomes with great beneficial effects for HCC patients.

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Conflict of interest The authors declare that they have no competing interest.

Appendix

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