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Mini-review

Tackling hepatocellular carcinoma with individual or combinatorial immunotherapy approaches

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ABSTRACT

Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer globally. Indeed, there is a single drug approved as first-line systemic therapy in advanced unresectable HCC, providing a very limited survival benefit. In earlier stages, 5-year survival rates after surgical and loco-regional therapies are extremely variable depending on the stage of disease.

Nevertheless, HCC is considered an immunogenic tumor arising in chronically inflamed livers.

In such a scenario, immunotherapy strategies for HCC, in particular combinations including cancer vaccines, may represent a key therapeutic tool to improve clinical outcome in HCC patients. However, a lot of improvement is needed given the disappointing results obtained so far.

1. Introduction

Hepatocellular carcinoma (HCC) represents the third leading cause of cancer death and every year more than 800,000 people die from HCC worldwide [1].

Surgery represents the standard treatment in the early stages of HCC, with a 5-year survival rate in 70% of treated patients and, when not applicable, loco-regional therapies represent a second line of therapy with highly variable 3 to 5-year survival rates [2,3]. The few approved systemic therapies in advanced unresectable HCC are the tyrosine-kinase inhibitors Sorafenib and Regorafenib (first and second line treatment, respectively) as well as the inhibitor of vascular endothelial growth factor receptors 1–3 Lenvatinib (as second line treatment). However, all of them provide only a very limited survival benefit [4–6]. Finally, the systemic chemotherapy has been reported to be unsuccessful in HCC patients because of the intrinsic chemoresistance of hepatocytes as well as the related severe toxicities [7].

Alternative to full dose chemotherapy, encouraging results have been obtained with low dose metronomic Capecitabine in HCC patients as second-line treatment after Sorafenib failure [8,9].

In order to improve the clinical outcome of HCC, immunotherapy may be a suitable strategy given that it is considered an immunogenic cancer because arising in chronically inflamed liver [10].

However, the highly immune suppressive HCC microenvironment may represent a major barrier to an effective anti-tumor activity elicited by immunotherapeutic interventions and needs to be balanced by immunoregulatory drugs. Therefore, a successful strategy requires the combination of different immunotherapy strategies to elicit an effective anti-cancer immune response, and chemotherapy/checkpoint inhibitors, to balance the immune suppressive tumor microenvironment (Fig. 1).

2. Immune microenvironment in hepatocellular carcinoma

Several cells are involved in the intra-hepatic tolerogenicity, which is relevant in host defense as well as in the maintenance of self-tolerance [11]. Liver sinusoidal endothelial cells (LSECs) express high levels of the inhibitory molecule program death receptor ligand 1 (PD-L1) and prevent immune responses against bacterial antigens coming from the gut [12]. Moreover, they also reduce the ability of dendritic cells (DCs) to activate T cells [13]. Kupffer cells (KCs) promote immunological tolerance in the liver by removing from the circulation gut-derived materials, producing inhibitory cytokines [14] as well as leading to proliferation of inhibitory $CD4^+$ regulatory T cells (Tregs) [15,16]. In addition, hepatic dendritic cells (HDCs) are poor stimulators of effector $CD4^+$ T cells expressing low levels of MHC II and co-stimulatory molecules and producing prostaglandin (PG) E2 which, in turn, increases IL-10 secretion and Tregs cells [17]. The physiological immune

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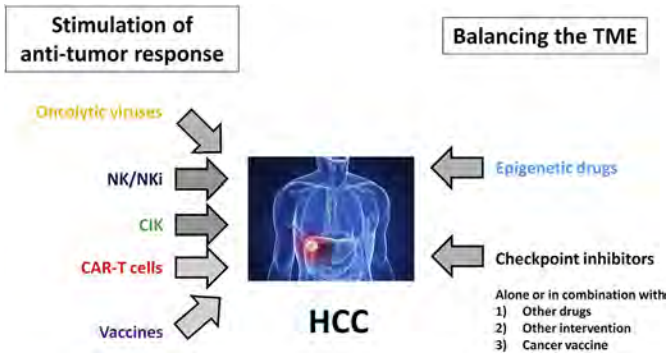

Fig. 1. Immunotherapies for HCC. All the different approaches tested for HCC in pre-clinical and clinical settings are indicated.

suppressive microenvironment of the liver tissue is further worsened during the formation and progression of HCC. In particular, fibrosis represents a physical barrier which prevents $CD8^+$ CTL infiltration into the intra-tumoral area and anti-TGFβ treatments have been proposed as anti-cancer therapy [18].

The high expression of immune checkpoint molecules on HCC tumor cells is an additional immune suppressive factor in HCC microenvironment. In particular, PD-L1 is mainly expressed on Kupffer cells but also on tumor cells and is associated with higher risk of cancer recurrence or metastasis and cancer-related death [19]. Additional inhibitory immune checkpoint molecules have been identified in HCC and correlated with poor prognosis. In particular, T-cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) is expressed on T cells and tumor-associated macrophages (TAM) infiltrating the tumor, correlating with worse prognosis [20].

Overall, the immune suppressive microenvironment is important to induce self-tolerance in the normal liver. However, this represents a strong obstacle for the development of an anti-tumor immunity as well as for the efficacy of immune-based therapeutic strategies. Therefore, approaches to modulate such unfavorable tumor microenvironment are needed in combination with immunotherapies for HCC.

3. Immunotherapy strategies for HCC

Therapeutic strategies for HCC based on immunotherapy are based on two principles: (1) to potentiate the already present anti-tumor immune responses; or (2) to stimulate new or different anti-tumor immune responses. Checkpoint inhibitors fall into the first category, unleashing a pre-existing immune reactivity to cancer which is blocked by inhibitory molecules expressed on tumor cells. Adoptive cell therapy as well as vaccines fall into the second category, by unmasking pre-existing and inducing de novo T cell responses to antigens expressed by HCC.

3.1. Immunotherapy trials based on immune checkpoint inhibitors

Immune checkpoints are expressed on the membrane of several immune cell types, with the physiological role of modulating the immune response. In particular, they have an immunosuppressive activity to prevent continuous and uncontrolled antigen-specific T cell responses, limiting collateral tissue damage. The immune checkpoints most studied in human cancer are cytotoxic T lymphocyte protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte activation gene 3 protein (LAG-3), B and T lymphocyte attenuator (BTLA), and T cell immunoglobulin and mucin-domain containing (TIM-3) [21,22].

To date, only CTLA-4 and PD-1/PD-L1 inhibitors have been evaluated in HCC (Table 1). The anti-CTLA-4 Tremelimumab, was the first molecule to be clinically evaluated for safety and tumor response in 20

Table 1
Clinical trials in HCC completed with reported results in publications.

DRUG NAME	TRIAL NR	PHASE	DOSE	SCHEDULE	PATIENTS	CR	PR	SD	PD	TTP	OS	6-MO PFS	1-Y PFS	6-MO OS	9-MO OS	1-Y OS	REF.
Tremelimumab	NCT01008358	II	15 mg/kg	every 90 dd x 4 doses	20	0%	18%	76%	6%	6,48 mo	8,2 mo	N/R	N/R	64%	N/R	43%	[23]
Tremelimumab + RFA/Chemoablation	NCT01853618	I/II	3.5 and 10 mg/kg	every 4 weeks × 6 doses + RFA	32	0%	26%	N/R	74%	7,4 mo	12,3 mo	58%	38%	57%	N/R	33%	[24]
Nivolumab	NCT01658878	I/II	0.1–10 mg/kg dose-escalation; 3 mg/kg dose-expansion	every 2 weeks	48 dose-esc; 214 dose-exp	1%	18%	45%	32%	N/R	N/R	N/R	N/R	83%	74%	N/R	[25]
Pembrolizumab	NCT02702414	II	200 mg	every 3 weeks	104	1%	16%	44%	33%	4,9 mo	12,9 mo	N/R	28%	N/R	N/R	54%	[27]

VACCINE	TRIAL NR	PHASE	DOSE	SCHEDULE	PATIENTS	CR	PR	SD	PD	TTP	OS	6-MO PFS	1-Y PFS	6-MO OS	9-MO OS	1-Y OS	REF.
DC pulsed w tumor lysate	N/R	N/R	5 doses	every week + 2–12 monthly	31	0%	13%	55%	32%	N/R	15 mo	N/R	N/R	N/R	N/R	40%	[48]
DC pulsed w tumor lysate	N/R	II	6 doses	every 3 weeks	39	0%	3%	15%	46%	N/R	5,6 mo	N/R	N/R	33%	N/R	11%	[49]
DC pulsed w HepG2 lysate	N/R	N/R	1 dose		30	0%	13%	60%	27%	N/R	7 mo	N/R	N/R	N/R	N/R	N/R	[50]
GPC3 peptide	N/R	I	0.3, 1, 3, 10, 30 mg		33	0%	3%	58%	39%	3,4 mo	9 mo	N/R	N/R	N/R	N/R	N/R	[54]
GPC3 peptide	N/R	II	N/R	N/R	57	N/R	N/R	N/R	N/R	N/R	20,1 mo	N/R	76%	N/R	N/R	N/R	[55]
MRP3 peptide	N/R	I	0.03–3.0 mg	every 8 weeks	12	0%	8%	75%	17%	N/R	14 mo	N/R	N/R	N/R	N/R	N/R	[56]
hTERT peptide	NCT00444782	II	0.56 mg	multiple admin	40	0%	0%	46%	50%	1,9 mo	12 mo	N/R	N/R	N/R	N/R	N/R	[59]

HCC patients infected with hepatitis C virus [23]. Partial response (PR) was observed only in 17.6% of the patients and stable disease (SD) was observed in 58.8% of patients. Time to progression was 6.48 months and the overall survival reached 8.2 months. A subsequent study by Duffy et al. combined standard treatments (i.e. tumor ablation utilizing RFA and TACE) with Tremelimumab, showing an improved infiltration of intratumoral effector $CD8^+$ T cells [24].

The anti-PD-1 Nivolumab was evaluated in HCC patients with various etiologies irrespective of any previous treatment with Sorafenib. Primary endpoints were safety, immunogenicity and antitumor activity (CheckMate 040). Complete response (CR) and partial response (PR) were reported in a minority of treated patients but the objective response rate was 20% in the dose-expansion phase [25]. Based on these results, Nivolumab is now included into the AASLD guideline on HCC as second line systemic therapy [26]. Similar results were observed in the KEYNOTE-224 trial which evaluated the anti-PD-1 Pembrolizumab in HCC patients who had progressed on Sorafenib [27]. However, the subsequent Phase III clinical trial KEYNOTE-240 did not meet its co-primary endpoints of overall survival (OS) and progression-free survival in patients with advanced HCC versus placebo [28]. Similarly, the phase III randomized control trial comparing Nivolumab with Sorafenib as first-line treatment in patients with advanced HCC (CheckMate 459) did not meet its primary endpoint of improved overall survival (OS) with Nivolumab as compared with Sorafenib for the treatment of patients with newly diagnosed, unresectable HCC [29]. Additional phase III trials is the ORIENT-32 study (NCT03794440) in China randomizing patients to a combination of Sintilimab (PD-1 inhibitor) and Bevacizumab (anti-VEGF antibody) versus a control arm of Sorafenib. The RATIONALE-301 study (NCT03412773) is a phase III trial randomizing patients to the PD-1 inhibitor tislelizumab monotherapy versus Sorafenib. Moreover, PD-1/PD-L1 inhibitors are currently evaluated in several clinical trials in combination with other treatments. Finally, a not yet enrolling phase II clinical trial at the University of Hawaii will evaluate the dual blockade of anti-TIM-3 and PD-1 in HCC (NCT03680508).

3.2. Adoptive cell therapy

This strategy is based on the concept of improving HCC outcomes by passively administering autologous immune cells following ex vivo cultivation [30]. NK cells, cytokine-induced killer (CIK) cells, TILs as well as chimeric antigen receptor T cells (CAR-T cells) have been studied in HCC to date.

3.2.1. NK and NKT cells

NK cells belong to the innate immune system and play a critical role in the host defense against solid tumors by direct killing of tumor cells as well as by releasing immune modulatory cytokines (reviewed in Ref. [31]). Reactivation of NK cells has been shown to be effective on HCC in preclinical studies [32], suggesting that HCC patients could benefit from enhancement of NK cells functionality [33]. 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) [34]. Moreover, two clinical trials were designed to assess NK cell therapy combined with liver resection (NCT02008929) or liver transplantation for HCC (NCT01147380) [35]. An ongoing trial is comparing a combination therapy of NK cell transfer with irreversible electroporation (IRE) vs. IRE alone (NCT03008343). NK T (NKT) cells are a heterogeneous group of T cells playing a role in regulating the anti-tumor immunity and exerting both immunosuppressive and immune activation functions [36,37]. A phase I clinical trial using autologous NKT cells to treat advanced HCC is now ongoing with no results available yet (NCT01801852).

3.2.2. Cytokine-induced killer cells

Cytokine-induced killer (CIK) cells are characterized by expression of both T cell and NK biomarkers and are generated from human peripheral blood mononuclear cells (PBMC) by treatment with IFN-γ, anti-CD3 antibody and IL-2 [38]. 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 [39–41]. In particular, immunotherapy based on CIK cells induces a significant increase in both overall survival (OS) and progression-free survival (PFS) of HCC patients. The largest study to date, involving 230 patients, evaluated CIK cell therapy as adjuvant to RFA, ethanol injection or curative resection, with an improvement of 14 months in recurrence free survival [42]. A systematic review and meta-analysis of CIK cell therapy in HCC in Asia reached similar conclusions [43].

3.2.3. T lymphocytes and CAR-T cells

The adoptive cell transfer strategy most experimented in cancer is based on T cells, including both native TILs and also CAR-T cells [44]. Administration of autologous TILs in 15 patients with HCC post-resection showed successful expansion in 88% of patients [45]. CAR-T cell therapy is now approved by the FDA approved the first two CAR-T cell therapies Kymriah® and Yescarta® for lymphoma in 2017. Since this approval, several trials into solid tumors have been conducted with contrasting results [44]. In order to have potentially successful CAR-T cells, the most important aspect is the selection of an appropriate antigen. In this framework, GPC-3 is highly expressed in HCC and is associated with poor prognosis. CAR-T cells specific to GPC-3 have been evaluated in patients with advanced HCC, portal vein invasion or extra hepatic metastases and reported at ASCO 2017 without a subsequent full publication [46]. Additional Phase I/II trials assessing CAR-T cells specific to GPC-3 are currently recruiting HCC patients (NCT03198546, NCT03130712, NCT02715362, NCT02723942) one trial is based on CAR-T cells specific to the EpCAM antigen (NCT03013712). TCR engineered T cells targeting alpha fetoprotein have been reported at ASCO 2016 without a subsequent full publication [47]. All these preliminary studies show feasibility and safety of the approach but efficacy needs to be assessed.

3.3. Cancer vaccine strategies

The number of human clinical trials published to date is extremely small. The first vaccines in HCC were based on whole tumor cell lysates. Autologous DCs loaded with autologous tumor lysate or a liver tumor cell line lysate (HepG2) were evaluated, showing safety and signs of efficacy in a percentage of patients. Increased median survival was between 4 and 12 months [48–50]. Subsequent cancer vaccines for HCC have been based on peptides representing only a single tumor associated antigen (TAA) but in most cases the frequency of antigen-specific T cells did not correlate with significant clinical outcome [51]. Peptides derived from alpha fetoprotein (AFP) were the first antigens selected for HCC-specific immunotherapy approaches, used alone or loaded onto autologous DCs. In both trials safety and immunogenicity were demonstrated, with an increased IFNγ-producing AFP-specific T cell responses to at least one of the peptides included in the mix [52,53]. A vaccine based on two peptides derived from glypican-3 (GPC3) was evaluated in advanced stage HCC patients in a phase I clinical trial, showing specific CTL responses in most of the vaccinated subjects correlating with overall survival (OS) [54]. Improved results were obtained combining the same vaccine with surgery [55]. Safety and immunogenicity of a MRP3-derived peptide has been shown in a phase I clinical trial, with partial response in one patient, stable disease in nine patients and disease progression in two patients [56]. Safety and immunogenicity of autologous DCs-pulsed with a peptide mix including MAGE-1, AFP and GPC3 cancer testis TAAs was evaluated in early phase clinical trials showing T cell responses against TAAs and a significant increase in the median time to progression (TTP) compared to

control group [57,58]. The telomerase-derived peptide GV1001 in combination with GM-CSF was evaluated in a phase II clinical trial in 40 advanced stage HCC patients. No GV1001 specific immune responses were detected after vaccination and none of the patients had a complete or partial response to treatment [59].

Novel shared "off-the-shelf" HCC-associated antigens have been identified within the HEPAVAC project (www.hepavac.eu) and a multi-epitope, multi-HLA peptide vaccine is currently evaluated for safety and immunogenicity in early-intermediate stage HCC patients undergoing surgical and/or loco-regional treatments (HepaVac-101 - NCT03203005) [60].

3.4. Immune modulatory approaches in HCC

In order to improve the efficacy of immunotherapies, immune modulatory approaches able to positively modulate the immune suppressive TME should be included in the therapeutic protocol.

3.4.1. Chemotherapy in HCC

Chemotherapy has been shown to enhance antitumor immunity, both at the maximum tolerate dose (MTD) and at low-dose metronomic schedule, killing of immunosuppressive cell populations (e.g. MDSCs and Tregs) as well as inducing immunogenic cell death (ICD) in cancer cells with release of danger signals able to polarize DCs and activate an anti-tumor T helper 1 (Th1) response. Moreover, they can modulate the expression of tumor antigens and immune checkpoint molecules, modifying the TME and improving the efficacy of immunotherapy treatments [61–63]. Systemic treatment with low-dose Cyclophosphamide in HCC patients has been shown to be safe and to decrease circulating regulatory T cells in peripheral blood, unmasking α-fetoprotein-specific $CD4^+$ T-cell responses [64].

3.4.2. Radiofrequency ablation in HCC

Alternatively, radiofrequency ablation (RFA) induces tumor destruction with release of TAAs as well as neoantigens, associated with significant intratumoral immune infiltrates and activation of immune response [65,66].

Both immune modulatory treatments have been evaluated in combination with cancer vaccines.

Cytotoxic drugs have been shown to improve anti-tumor effects of cancer vaccines in pre-clinical as well as in clinical settings counteracting the immune-suppression, enhancing cross-presentation of tumor antigens and increasing the number of effector cells in the tumor microenvironment [67–69].

Combination of RFA and cancer vaccine has been evaluated in preclinical experimental settings, showing enhancement of antitumor immunity [70,71], and in a single clinical trial showing an improved recurrence rate in HCC patients [72].

An additional strategy is represented by combination of cancer vaccines and checkpoint inhibitors, which has shown significant enhancement of anti-tumor response associated with increased infiltration of effector $CD8^+$ T cell in pre-clinical as well as in clinical settings [69,73–77].

However, the latter combinatorial strategy has not been evaluated in HCC yet.

3.4.3. Epigenetic drugs in HCC

Epigenetic modifications induce an overall silencing of gene expression playing a major role in the cancer development and progression, impairing immunogenicity and immune recognition of cancer cells. The epigenetic mechanisms involved in cancer cells include hypermethylation, mediated by DNA methyltransferase (DNMTs) enzymes, and histone deacetylation, mediated by histone deacetylase (HDACs) enzymes. Both mechanisms induce a loss of function of several genes relevant to cancer initiation and progression (i.e. tumor suppressor genes) or immunological function (i.e. MHC class-I expression). The final effect is an efficient tumor cell growth and the escape from the host's immune recognition [78].

Given the relevance of epigenetic alterations in tumorigenesis, specific anti-cancer therapies have been developed and tested in preclinical and clinical settings. It has been shown that epigenetic drugs have not only a direct antitumor effect by reducing cell proliferation and viability, inducing cellular apoptosis, but they can simultaneously modify the antitumor immunity. Indeed they modulate antigen expression and the machinery responsible for their presentation and recognition by T-cells [79]. Moreover, they may also directly act on the immune system, activating effector cells and inhibiting immunosuppressive mechanisms [80,81]. Furthermore, DNA methyltransferase inhibitors (DNMTi) have been shown to derepress expression of cancer–testis antigens and human endogenous retroviruses, which are suppressed by DNA methylation in most somatic cells. Activation of these cancer–testis genes and repetitive sequences can potentially give rise to the presentation of neoantigens by treated cells, thus increasing visibility to host's immune surveillance [82].

Both DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) are approved by FDA for the treatment of hematologic malignancies. In particular, the HDACi azacytidine (AZA) and decitabine (DAC) have been approved for myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) [83,84]. The HDACi Vorinostat, Romidepsin, Panobinostat have been approved for cutaneous T-cell lymphoma (CTCL) and Belinostat for peripheral T-cell lymphoma (PTCL), reviewed in Ref. [85].

Recently, DNMT and HDAC inhibitors have been introduced in preclinical studies of HCC. The HDACi Belinostat has been shown to improve therapeutic efficacy of the anti-CTLA4 checkpoint inhibitor and their combination reduces tumor growth and prolongs survival in a mice model of HCC, associated to an increased tumor infiltration of M1 macrophages and decreased Tregs [86].

The DNMTi 5-Azacytidine (5-AZA) induces the expression of neoantigens on cell surface of HCC cell lines, which are normally expressed in low dose. Moreover, it has been demonstrated that the combination of 5-AZA and anti-PDL-1 decreases tumor size and weight as well as increases T-lymphocyte infiltration, confirming that epigenetic modulation could be a novel potential strategy to augment immunotherapy for HCC [87]. Interestingly, DNMTis have been shown to sensitizing HCC cells to Sorafenib, the only systemic drug approved as first line treatment in inoperable HCC patients. Decitabine, alone and in combination with chemo- or adoptive immunotherapy, is able to resensitize resistant tumor cells to Sorafenib and to decrease colony formation ability [88].

Only few clinical studies have been conducted so far in patients with hepatocellular carcinoma. In a Phase I/II trial, Mei Q et al. have shown the safety and prolonged PFS and OS by low-dose decitabine in heavily pre-treated patients with advanced HCC (NCT01799083) [89]. Similarly, the trial conducted by Yeo W et al. showed that 42% of enrolled patients with unresectable HCC achieved stable disease and increased OS after treatment with Belinostat (NCT00321594) [90]. All these preclinical and limited clinical results suggest the promising role of epigenetic drugs in combination with conventional therapy in the treatment of HCC.

3.5. Oncolytic viruses

Oncolytic viruses (OVs) have the biological property of infecting and killing cancer cells without damaging healthy tissue. OVs are mostly derived from Reovirus (RV), Vaccinia virus (VV), Herpesvirus and Adenovirus which, upon genomic editing, acquire the highest cancer-specific selectivity [91]. Most of the preclinical studies in HCC use adenoviruses and vesicular stomatitis virus (VSV) as vector, due to their natural ability to kill or target HCC cells [92,93]. However, other viruses have been used as oncolytic viruses in HCC in many pre-clinical studies (reviewed in Ref. [94]). The talimogene laherparepvec (T-VEC),

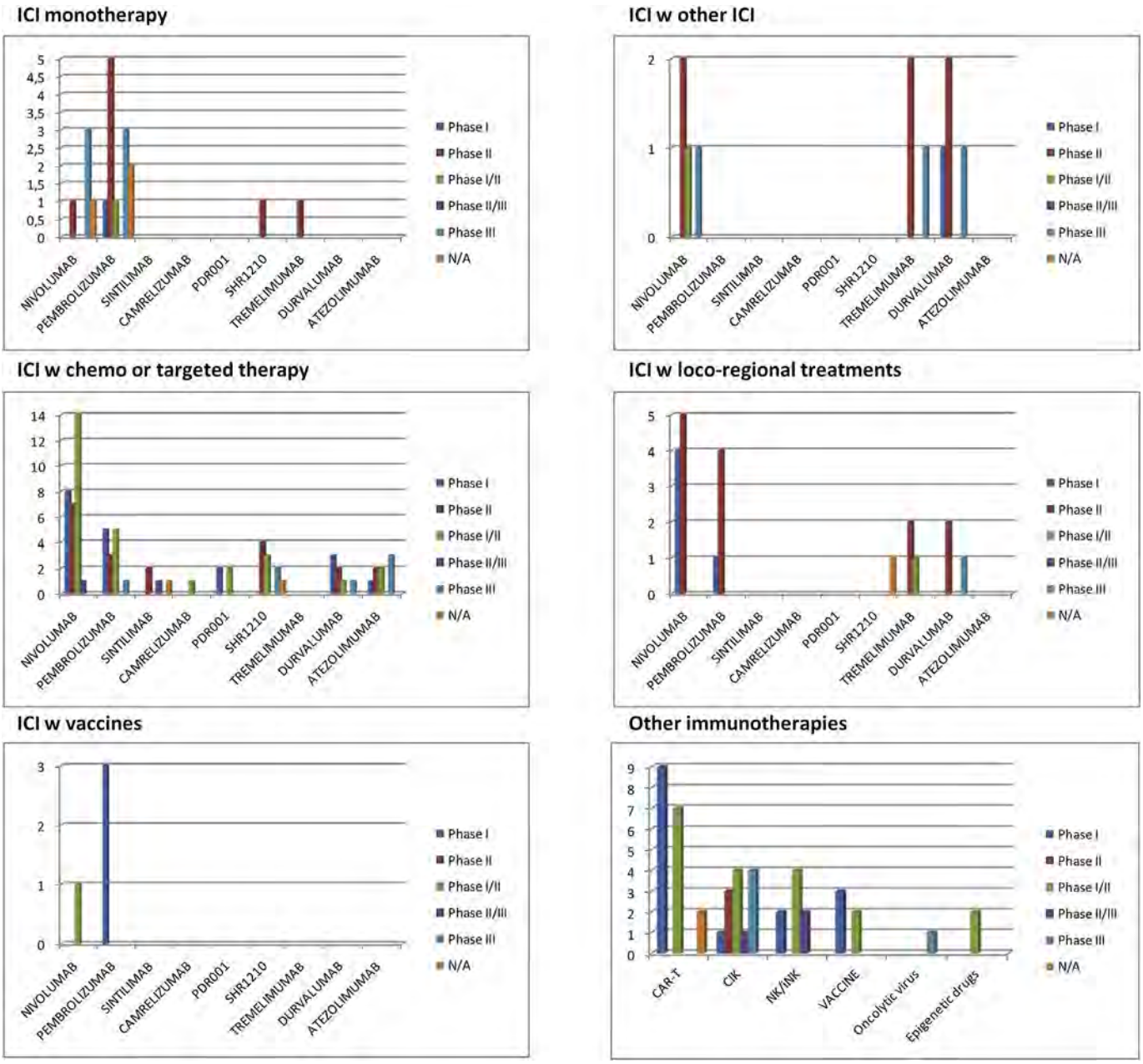

Fig. 2. Currently ongoing clinical trials in HCC. Clinical trials involving ICI are divided according the protocol (monotherapy vs. combination with other agents). All other immunotherapies are displayed in a single panel.

a modified herpes simplex virus type 1, is the only oncolytic virus to date approved by FDA for treatment of melanoma based on the improved objective response to treatment and longer overall survival [95]. A phase I study in patients with HCC and liver metastases where T-VEC is injected directly into the liver lesions is currently underway (NCT02509507). Alternatively, pexastimogene devacirepvec (Pexa-Vec) is an oncolytic virus derived from the vaccinia virus which has been shown to be well tolerated and to improve overall survival in patients with unresectable HCC [96], although such results were not duplicated in patients who failed to Sorafenib [97].

Several studies are underway investigating Pexa-Vec in HCC patients. A phase III clinical trial was recruiting patients with advanced HCC who have not received prior systemic therapy to compare Pexa-Vec followed by Sorafenib to Sorafenib alone (NCT02562755). Such a trial has been recently prematurely terminated after failing an interim futility analysis, revealing that the study was unlikely to meet its primary objective [98]. A phase IIa clinical trial has evaluated Pexa-Vec in patients with advanced HCC either Sorafenib-naïve (NCT01636284) or who failed to Sorafenib therapy (NCT01387555). Finally, a phase I/II clinical trial is evaluating Pexa-Vec in combination with Nivolumab (NCT03071094).

4. Mutational landscape and neoantigen in HCC

In the quest for more specific target antigens for cancer immunotherapies, identification of tumor-specific mutated neoantigens deriving from somatic genetic mutations generated during cancer formation and progression represent the ultimate frontier. Indeed, tumor associated antigens (TAAs) may be present at low level also on normal cells and, consequently, may be subject to both central and peripheral tolerance mechanisms [99]. Consequently, the best non-self immunological target is represented by real tumor-specific antigens

deriving from public or personal mutations in cancer cells [100]. The efficacy of checkpoint inhibitors has been shown to strongly correlate with the number of predicted neoantigens target of the infiltrating T cells [101,102]. However, identification of naturally presented neoantigens by mass spectrometry has proven to be extremely inefficient [103–105], therefore tumor neoantigens are predicted and validated through bioinformatics and experimental pipelines for which a general consensus has not achieved yet. Indeed, prediction of mutated neoantigens should account on high affinity to HLA molecules, and in particular 10 times higher than the affinity of the corresponding wild type epitope (differential agretopicity index, DAI > 10), but also on the lack of sequence homology with any wild type cellular self antigens [106–108]. Furthermore, if such neoantigens show homology with pathogen-derived epitopes, the pre-existing pathogen-specific immunity will respond faster and stronger to such neoantigens, resulting in a more efficient control of the tumor evolution and, consequently, in a better clinical outcome [106,109]. Nevertheless, immunological validation is absolutely required to confirm the effective “quality” of the predicted antigens.

Among others, HCC ranks as a medium variable tumor, with an average mutational burden of 5 somatic mutations per Mb [110]. Our group recently reported the first neoantigen discovery in HCC, describing that HCC patients with improved survival showed the highest number of mutated antigens with high affinity to HLA, no or low sequence homology with corresponding wt peptides and homology with pathogen-derived antigens [108].

5. Conclusions

Hepatocellular carcinoma is challenging to treat and, besides surgical and loco-regional interventions, Sorafenib is the only systemic drug is approved as first line treatment in inoperable HCC patients. Immunotherapy may represent a significant improvement for HCC treatment but it has not been extensively explored yet compared to other cancers (Fig. 2; Suppl. Table 1). Anti-PD-1 has proven efficacy as second line systemic therapy but has not provided improved overall survival compared to Sorafenib as first-line treatment. Epigenetic drugs hold promise to be one of the tools to be implemented in the HCC therapy. Results from few clinical trials evaluating adoptive T cell therapy are awaited. Cancer vaccines are currently developed and evaluated in clinical trials based on new TAAs as well as personalized mutated neoantigens. Expanding observations on all these strategies individually and in combination, will be the main path allowing building a relevant knowledge for improving clinical outcome in HCC.

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Declaration of competing interest

The authors declare no potential conflicts of interest.

Appendix A. Supplementary data

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