



Review

Immunotherapy of hepatocellular carcinoma

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1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world with a 5 year survival rate of less than 5% and incidence of at least one million new patients per year [1]. For a small group of HCC patients, surgical or local ablative therapy might be suitable. However, these therapies are limited by tumor size, hepatic functional reserve and intra-hepatic metastases. Therefore, for the majority of patients with advanced disease, these treatments with curative intent are no longer possible [2]. Transarterial chemoembolization (TACE) prolongs survival of patients not eligible for surgical resection, liver transplantation or tumor ablation, but only patients with adequate liver function can be treated by this procedure [3,4]. Systemic chemotherapy is only moderately tolerated due to the underlying liver cirrhosis in most patients with HCC and not effective [5]. Therefore, alternative treatments such as immunotherapy are currently being evaluated in patients with advanced HCC according to international guidelines (EASL and AASLD) [6,7].

A retrospective analysis of 163 surgically resected HCCs demonstrated that infiltration of tumors with lymphocytes resulted in a better prognosis [8]. A number of studies have also shown that immunotherapy might become a potential therapeutic option for patients with HCC [9]. Advances in the understanding of basic tumor immunology have opened new paths to treating certain malignant tumors and may also be the case for patients with HCC in the near future. Here, we describe recent immunological findings in patients with HCC and report about initial promising results from the first clinical immunotherapy trials in HCC.

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2. Immunobiology of cancer vaccines

The requirement for an immune-based strategy against cancer is the induction of an effective tumor-specific response to break tolerance to the tumor and generate long lasting anti-tumor immunity. To achieve this goal, a variety of strategies both in preclinical models and in clinical trials are currently being investigated. There have been numerous immunotherapeutic approaches to mobilize or manipulate the immune system. These include non-specific activation of the immune system such as use of cytokines as well as antigen-specific immunotherapy such as use of autologous and allogeneic tumor cells (engineered to secrete cytokines), peptides, proteins and DNA vaccines as well as tumor-specific antibodies.

Until today most immunotherapeutic approaches are based on the generation of tumor-specific CD8⁺ T cells, which recognize 8–11 amino acid long peptides derived from intracellular proteins, and presented on MHC class I complexes. These proteins can be over-expressed, mutated, or virally induced in tumors and therefore represent potential targets for effector cells. While it is also possible to adoptively transfer antigen-specific T cells into patients [10], most cancer vaccines rely on the generation of antigen-specific T cells *in vivo*.

Different approaches can be used to achieve this goal (Fig. 1). MHC class I restricted peptides, either used alone or in combination with cytokines such as GM-CSF, can be used as a cancer vaccine. They are injected subcutaneously, where they bind to MHC class I molecules from local professional antigen-presenting cells such as dendritic cells (DCs). DCs express high levels of co-stimulatory molecules, secrete cytokines and can present antigenic peptides to T cells to induce effector responses. Alternatively, whole tumor cells can serve as vaccines. In contrast to peptide vaccines, whole tumor cells have to be processed by DCs. Whole tumor cells have the advantage that they express all potential tumor

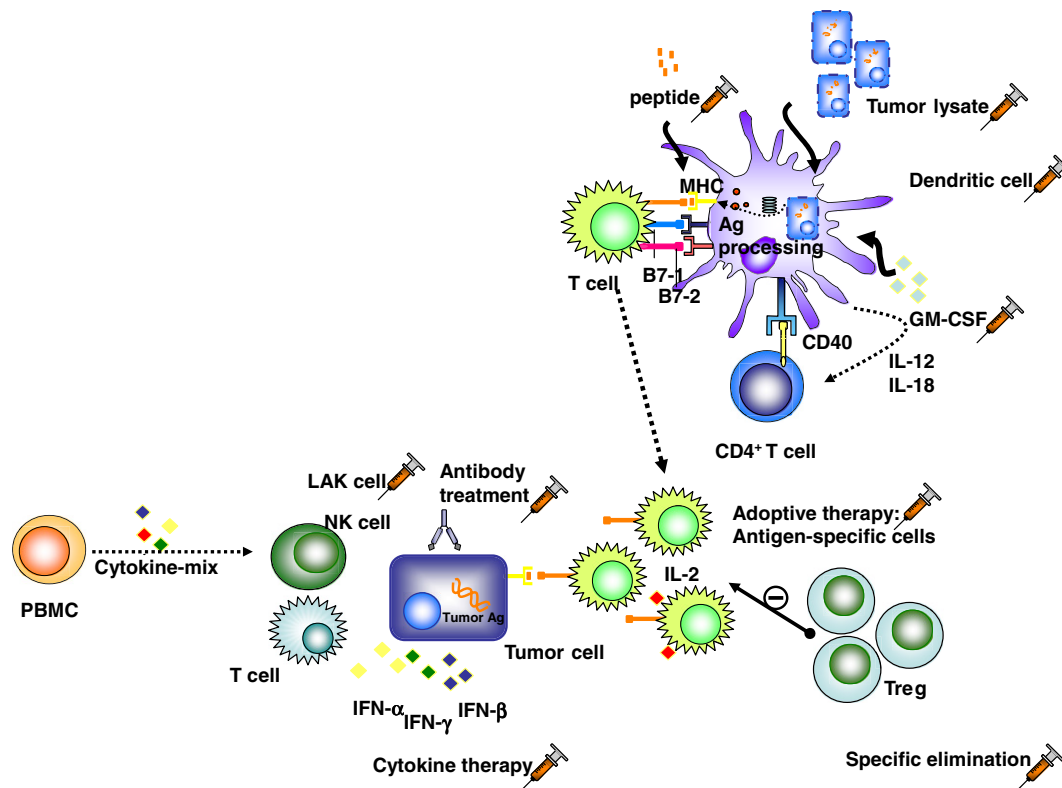


Fig. 1. Immunotherapeutic approaches to cancer. Tumor cells can be targeted by different effector mechanisms. Lymphokine activated killer cells can be generated from peripheral blood and directly use to treat tumors. Alternatively, systemic cytokine treatment can be performed. In contrast tumor-specific effectors consist of either humoral responses (antibodies) or cellular responses such as cytotoxic CD8⁺ T cells. Tumor-specific T cells can be generated *in vitro* and injected into the patient (adoptive T cell therapy) or *in vivo*. Mature DCs express co-stimulatory molecules such as B7-1 and B7-2 to enhance T-cell activation. DCs secrete IL-12 and IL-18 to promote T helper CD4⁺ T cell responses and cytotoxic CD8⁺ T cells. Activated CD4⁺ T cells express CD40 ligand, which further stimulates DC maturation through CD40 signaling. DCs are either intratumorally injected, or loaded with the corresponding tumor antigen. This can be achieved *in vitro* or *in vivo* by injection of antigenic peptides or tumor lysate. Finally, elimination of regulatory T cells can lead to an increased immune response *in vivo*.

antigens in contrast to a peptide vaccine, where only one or a few antigens are used for vaccination. Therefore, whole tumor cells can activate CD4⁺ T helper cell responses as well as CD8⁺ T cells. CD4⁺ T cells recognize peptides presented on MHC class II molecules on the surface of professional antigen-presenting cells such as dendritic cells. It has been shown that induction of tumor-specific CD4⁺ T cells is crucial not only to activate CD8⁺ T cells, but also to mediate anti-tumor effector functions [11,12].

3. Tumor antigens in HCC

The identification of tumor antigens represents an important step for the development of potent cancer vaccines. These antigens can be either tumor-specific, which are expressed exclusively in tumor tissue; tumor-associated or cancer-testis antigens, which are over-expressed in tumors but are also detected in placental trophoblasts and testicular germ cells; and differentiation antigens which are over-expressed in tumors and are

also found in normal tissue of origin [13,14]. Alpha-feto-protein (AFP), which is expressed in up to 80% of all HCC tumors, but not in normal adult tissue, represents a classical tumor antigen for HCC. AFP has been a target for immunotherapy of HCC in several studies. Different MHC class I and II restricted epitopes from AFP have been identified [15–19]. Recently, PTTG1, or pituitary tumor transforming gene 1, which plays a role in promoting tumor angiogenesis was shown to be over-expressed in HCC [20].

Our knowledge of most other HCC-specific antigens comes mainly from different tumors such as melanoma, where tumor antigens were initially identified but later their expression was also detected in HCC. Cancer-testis antigens are exclusively found in tumors with the exception of testis and therefore do not encompass the risk of autoimmune reactions during immunotherapy [21]. So far the best-characterized cancer-testis antigens in HCC are the MAGE antigens. Table 1 summarizes results from numerous studies analyzing the expression of different tumor antigens in HCC. As shown, up to 80% of all HCC express the MAGE-1 antigen, making

Table 1
Tumor antigens in HCC

Antigen	HCC tumor (%)	References
MAGE-A1	19–80	[24,26,39,105–113]
MAGE-A2	30–34	[110,111]
MAGE-A3	24–70	[24,26,39,106,107,109–111,113]
MAGE-A4	5–20	[24,26,107,110]
MAGE-A6	30	[111]
MAGE-A8	46	[111]
MAGE-A10	19–37	[24–26,111]
MAGE-A11	30	[111]
MAGE-A12	30	[111]
SSX-1	38–80	[24,28,105,107]
SSX-2	10–47	[24–26,28,107,114]
SSX-4	10–73	[24,28,107]
SSX-5	30–47	[24,28,114]
NY-ESO-1	13–51	[22–28,115]
MAGE-B1	45–52	[24,106]
MAGE-B2	60–62	[24,106]
MAGE-C1	48	[24]
MAGE-C2	68	[24]
BAGE	21	[109]
LAGE-1	16–25	[25,115]
SCP-1	33	[24,26]
HCA 90	100	[116]
HCA 519	100	[116]
HCA 520	50	[116]
HCA 587	56–70	[105,116]
HCA 661	29	[116]
CTp11	63	[105]
GAGE	38	[107]
GAGE 1-6	36	[109]
GAGE 1-2	30	[109]
TES14/SCP1	29	[107]
TES85	19	[107]
TSPY	29	[117]
GPC3	Most	[40,118]
hTERT	80–90	[119]

More Ag are described in [120,121].

this an interesting target for future immunotherapeutic treatment of HCC. NY-ESO-1, a different cancer-testis antigen, was one of the early antigens identified by serological analysis of recombinant cDNA expression libraries using tumor mRNA and autologous serum from a patient with esophageal squamous cell carcinoma [22]. Others and we have shown that NY-ESO-1 is expressed in up to 50% of HCC [23–28]. A more recent study has found a correlation between NY-ESO-1 expression and an early tumor stage [24]. Until today the exact function of this antigen has remained unknown. However, due to its high immunogenicity, a number of clinical trials have been initiated based on the NY-ESO-1 antigen [29–31]. Another antigen, the catalytic telomerase subunit (hTERT), is expressed by more than 85% of human cancers and represents an attractive tumor antigen for a variety of tumors [32]. It is also up-regulated in the multi-step process of hepatocarcinogenesis [33] and has been suggested as a novel tumor marker for HCC due to its up-regulation

in the tumor and the possibility to detect it in serum [34]. This makes hTERT another interesting candidate tumor antigen for HCC [35].

4. Tumor-specific humoral and cellular immune responses in HCC patients

An ideal tumor-specific target should generate tumor-specific humoral and cellular immune responses in cancer patients. AFP has been a serum marker for HCC and has been also used as a target in antibody-based therapies [36,37]. It has been shown that AFP-specific CD8⁺ T cell lines can be generated from patients with HCC [15] but also from patients with liver cirrhosis without HCC [38]. The clinical significance of NY-ESO-1 as a tumor antigen in HCC was demonstrated, when NY-ESO-1-specific immune responses were analyzed. We have shown that more than 50% of HCC patients with NY-ESO-1 expressing tumors have specific antibody responses to this antigen [23]. Interestingly, no NY-ESO-1 specific antibodies were detected in serum from a patient initially positive for NY-ESO-1 antibodies after curative resection of his tumor, while in those cases where tumors progressed, NY-ESO-1 antibody titers increased. Additionally, NY-ESO-1 specific CD8⁺ and CD4⁺ T cells were only detected in patients with NY-ESO-1 expressing HCC [23]. A different study demonstrated that NY-ESO-1 specific CD8⁺ T cells could recognize hepatoma cells transfected with the NY-ESO-1 cDNA [27]. Of the other cancer-testis antigens, there has been SSX-2 and MAGE-10-specific CD8⁺ T cells in selected patients, which were able to kill tumor cells *in vitro* [25]. Another study describes the presence of MAGE-1 and MAGE-3 specific CD8⁺ T cells in HCC infiltrating lymphocytes [39]. Recently a new tumor antigen, glypican-3 (GPC3), has been identified, for which reactive T cells have been isolated from patients with HCC [40].

5. Tumor escape mechanisms

Tumors have developed several escape mechanisms from the immune system to inhibit anti-tumor responses. Production of immunosuppressive factors, increase in regulatory T cells, down-regulation of tumor antigens and MHC molecules are all mechanisms by which tumor cells escape from immune recognition [41,42]. There is clear evidence that the development of HCC can lead to suppression of tumor-specific immune responses. In particular, we have demonstrated that HCC progresses despite tumor-specific cellular and humoral immune responses detected in more than 50% of HCC patients [23]. Given that HCC tumors still progress albeit the presence of antigen-specific T cells, it raises concern

about the efficacy of immunotherapy for the treatment of this tumor. As it will be shown below, the development of HCC leads to a number of different immunological mechanisms resulting in suppression of tumor-specific immune response in HCC patients.

6. Dendritic cells

Dendritic cells (DCs) are the most potent antigen-presenting cells (APC) unique in their ability to efficiently prime both CD4⁺ and CD8⁺ cytotoxic T cells. The potent ability of DCs to activate as well as inhibit immune responses makes them one of the best candidates for tumor vaccines. DCs can either be loaded with tumor cells *ex vivo* and used as a vaccine or injected directly into tumors, where they pick up the antigen and present it to T cells to generate an effective anti-tumor immune response (Fig. 1).

It has been suggested that impaired function of DCs might be an important factor in the escape of the tumor from the immune control in cancer patients [43]. Several studies have shown that in peripheral blood of cancer patients, the number of dendritic cells is significantly reduced [44,45]. Furthermore, others and we have shown that dendritic cells from cancer patients are mainly immature and cannot stimulate T cells [46–48]. The abnormal differentiation of dendritic cells which results in a decrease in DC number and immature phenotype is proposed to be influenced by tumor derived factors, such as VEGF [49–51], M-CSF, IL-6 [52–54], and IL-10 expression [55–57]. Finally, AFP has also been identified as a possible inhibitor of DC function [58].

In this review, we describe some of the clinical trials using dendritic cells as vaccines in HCC patients (see below). However, it should be noted that a DC-based therapeutic approach is a very complex process depending on several factors; type and quality of DCs, manner of antigen loading, dose and route of vaccination. In addition, DCs have to be prepared for every individual patient making this approach only feasible for very selected patient groups.

7. CD4⁺ CD25⁺ regulatory T cells in HCC

Regulatory T cells were initially described in the early 1970s as suppressive T cells [59]. A growing number of observations from the past few years have demonstrated that regulatory-T-cell-mediated immunosuppression is one of the central tumor immune-evasion mechanisms and may be the main obstacle of successful tumor immunotherapy [60]. CD4⁺ CD25⁺ regulatory T cells mediate peripheral tolerance by suppressing self-antigen-reactive T cells [61,62]. We and others have found an increase in frequency of CD4⁺ CD25⁺ regulatory T

cells in tumors as well as in malignant ascites and tumors from HCC patients [63–65].

A number of different mechanisms have been suggested as to explain the increase in the number of CD4⁺ CD25⁺ regulatory T cells in cancer patients [60]. Tumor environmental factors such as vascular endothelial growth factor, IL-10 and transforming growth factor- β , which all are elevated in HCC [66–68] might induce regulatory T cells. It also been shown that maturation status of dendritic cells can also induce a regulatory phenotype in T cells [69].

Several groups are attempting to determine the influence of attenuating or suppressing regulatory T cell activity in cancer immunotherapy. Depletion of regulatory T cells using anti-CD25 mAbs has shown substantial anti-tumor effects in murine tumor models [70,71,153]. Administration of regulatory T cell inhibiting agents such as cyclophosphamide has shown to be effective in enhancing the anti-tumor effects in preclinical models [72]. Renal cancer patients, who underwent depletion of regulatory T cells before receiving a kidney cancer vaccine, had a 1000-fold expansion of tumor-specific T cells [73]. It remains to be seen whether depletion of regulatory T cells in HCC patients would lead to an enhanced anti-tumor immune response or not.

8. Antibody based therapies

Anticancer antibodies can be classified according to their mechanism of action [74]. AFP-specific antibodies, which are conjugated to toxins or radioactive compounds, are designed to bind tumor cells specifically and cause tumor cell death and have been evaluated for more than 20 years [36,37]. Monoclonal antibodies that interfere with signals transmitted from growth factor receptors such as EGFR and those inhibiting angiogenesis are currently being evaluated for treatment of HCC [75–77].

Other antibodies are specific for molecules on the surface of immune cells, where they either provide activating signals to lymphocytes and antigen-presenting cells or block the action of inhibitory receptors. 4-1BB (CD137) is a surface glycoprotein, which belongs to the TNF receptor family and is expressed by activated T cells, NK and dendritic cells. Ligation of 4-1BB *in vitro* provides costimulation for lymphocyte proliferation and cytokine secretion. It has been shown that treatment of tumor bearing mice with anti-4-1BB antibody causes tumor regression [78]. Interestingly, analysis of tumor-infiltrating lymphocytes from HCC patients indicated 4-1BB expression on CD4⁺ and CD8⁺ T cells, which was absent on lymphocytes from their peripheral blood [79]. These findings suggest that treatment with anti-4-1BB antibody might become a valuable approach to enhance tumor-specific immune responses in HCC

patients. A different approach targeting another co-stimulatory molecule, B7H-1, has demonstrated first promising results in a murine hepatocellular carcinoma model [80]. These results need to be further confirmed in HCC patients.

CD40, another member of the TNF receptor family, has a fundamental role in shaping both cellular and humoral immune responses [81]. CD40 is expressed on B cells, DCs and macrophages, but can also be found on tumor cells including HCC [82]. Its specific ligand, CD40L, is expressed by activated T-helper cells in a highly restricted manner [83]. Ligation of CD40 on the surface of dendritic cells greatly increases their antigen-presentation and co-stimulatory capacity. This can either be achieved through the help of CD4⁺ T cells expressing CD40L or by a CD40 activating antibody [84]. Treatment of Morris hepatoma bearing ACI-rats with an activating anti-CD40 antibody increased the number of tumor infiltrating T and NK cells and impaired tumor growth [85].

9. Human clinical trials

Due to the poor prognosis of patients with advanced HCC and the lack of efficient therapeutic options, it is clear that immunotherapy is a viable option for treatment of HCC. A number of immunotherapeutic options have been evaluated in patients with HCC and are summarized in Table 2. Most of these trials are non-randomized phase I or II studies with less than 30 patients enrolled. Therefore, these studies mainly demonstrate feasibility and safety, but still lack definite proof of the efficacy of immunotherapy for HCC. It is important to note that the majority of HCC patients suffer from liver cirrhosis and impaired liver function. This causes increased toxicity of conventional chemotherapy and makes immunotherapy a promising therapeutic option for this group of patients.

In 2000, Takayama and colleagues have published the results of a randomized trial comparing adoptive immunotherapy using IL-2 and anti-CD3 stimulated autologous PBMC after curative resection vs. resection of HCC alone. Adoptive immunotherapy reduced tumor recurrence in HCC patients by 41% [86]. This study is the first important example of the potential of cancer immunotherapy for the treatment of HCC. Other trials have tested the combination of adoptive immunotherapy plus systemic chemotherapy in small trials [87,88] or adoptive cell therapy alone [89] for the treatment of HCC. While these trials have been too small to evaluate the efficacy of the treatment, they clearly demonstrate the feasibility and safety of this therapeutic approach.

Several studies have shown the potential for a DC-based immunotherapy for HCC patients. It has been shown that dendritic cells from HCC patients can be

transduced using an AFP expressing adenovirus in order to stimulate AFP-specific immune responses [90]. Results from the first phase I/II clinical trial using AFP peptide-pulsed dendritic cells were recently reported. Significant AFP-specific T cell responses were detected in six patients after vaccination [91].

In addition, dendritic cells loaded with RNA cells from HepG2 tumor cells were also able to generate anti-hepatocellular carcinoma T cells [92]. Loading of dendritic cells with Hsp70-peptide complexes derived from human HCC cells resulted in maturation of dendritic cells which in turn stimulated proliferation of autologous HCC-specific cytotoxic T lymphocytes (CTLs) [93].

Two small pilot studies have studied intratumoral injection of dendritic cells in HCC patients. Intratumoral injection of DCs relies on the ability of dendritic cells to capture antigens from the tumor cells and transport them to draining lymph nodes, where the tumor antigen is presented to T cells. This approach has the advantage that neither autologous tumor material is needed nor the vaccine is restricted to a single antigen [94,95]. In addition, trials using dendritic cells pulsed with autologous HCC tumors or tumor cell lines have just been initiated [96]. These studies have mainly demonstrated the safety and feasibility of this albeit cumbersome method. One phase I study showed the safety and feasibility of autologous DC in 10 HCC patients [97]. Another study has also used DC pulsed with tumor lysate and demonstrated that about 10% of the HCC patients showed a partial response confirming the feasibility and safety of the DC vaccination in these patients [98].

In 2004, results from a randomized phase II trial using a mixture of GM-CSF, IL-2, formalin-fixed autologous tumor material, Calmette-Guérin was published. The vaccine was well tolerated and no major side effects occurred. More than 85% of all patients were HBV positive and approximately 50% of the patients had liver cirrhosis. Progression free survival and overall survival were significantly improved in the vaccinated group with an overall survival of 18/19 in the vaccinated group and 13/21 in the control group after 24 months [99].

Antigen-specific vaccinations in HCC patients have been tested using four immunodominant AFP peptides emulsified in Montanide ISA-51 in a small phase I clinical trial including six patients with advanced HCC [100]. Although, not surprisingly, no clinical responses or AFP serum level decreases were detected in these patients, detailed immunomonitoring clearly demonstrated induction of AFP-specific CD8⁺ T cells in the majority of the patients. In contrast to dendritic cell based therapies, antigen-specific vaccines do not have to be prepared for every individual patient if shared tumor antigens are used, which makes production of the vaccine much less complex and expensive.

Interestingly, a recent study analyzing T cell-specific immune responses in 20 HCC patients undergoing

Table 2
Immunotherapy trials in HCC since 1990

Year	Strategy	# of patients	Response	Reference
1990	Advanced HCC: IFN- γ treatment			[122]
	Advanced HCC IFN- α 2b	30	7% response rate	[123]
1991	Adjuvant: doxorubicin \pm intra-arterial injection of LAK (d 10,14 and 21) + IL-2	30 (24 valuable)	Recurrence rate: 8,3% adriamycin + LAK 50% adriamycin	[87]
	Advanced HCC: indium-111-labeled tumor-infiltrating lymphocytes (111In-TIL) intrahepatic arterial bolus	3 (1 HCC)	Feasibility	[124]
1993	Advanced HCC: IFN- α	71	Median survival: 14.5 weeks with treatment 7.5 weeks without treatment	[125]
	Advanced HCC: mitoxantrone + IFN- β	40	Median survival: 8 months	[126]
	Advanced HCC: doxorubicin + IFN- α	31	Response rate: 3%	[127]
1994	Advanced HCC: melatonin + IL-2	14	Response rate: 36%	[128]
	Advanced HCC: doxorubicin + IFN- α 2b	21	Response rate: 14%	[129]
	Advanced HCC: doxorubicin + IFN- γ	30	Response rate: 7%	[130]
1995	Adjuvant: pre- and post-operative targeting loco regional chemotherapy-immunotherapy	40	No adjuvant therapy: 40% recurrence rate. With adjuvant therapy: 0% recurrence rate (3–27 months follow-up)	[131]
1995	Advanced HCC: transarterial intrahepatic: IL-2 + IFN- γ + lipiodol	20	Median survival: 18 months	[132]
	Adjuvant: intra-arterial injection of adriamycin \pm IL-2 + LAK	24	No difference in survival rate	[88]
	Advanced HCC: doxorubicin + IFN- α + tamoxifen + desferrioxamine + ASS	7	No major side effects	[133]
	Advanced HCC: epirubicin + IFN- α 2b	31	Median survival: 9.5 months	[134]
1996	Advanced HCC: 5-FU + IFN- α	10	Median survival: 10 months (0% response rate)	[135]
	Advanced HCC: LAK vs. CTL	8 and 18	LAK group: 0/8 CR or PR CTL group: 3/18 CR, 2/18 PR	[136]
	Advanced HCC: cisplatin + IFN- α vs. no treatment	30/26	Median survival: 33 vs.14.0 weeks	[137]
1997	Adjuvant: TILs	12	Reduced recurrence rate	[138]
1998	Advanced HCC: TACE + α -1-thymosin	12	Comparison with historic control shows survival benefit	[139]
2000	Adjuvant: activated autologous lymphocytes vs. no treatment	75/75	Significantly longer recurrence-free survival after transfer of activated lymphocytes	[86]
	Advanced HCC: IFN- α 2b vs. no treatment	28/30	No survival difference	[140]
	Adjuvant: IFN- β vs. no treatment	10/10	Recurrence rate after 24 months: 0% with IFN- β 100% without treatment	[141]
2001	Adjuvant: IFN- α vs. no treatment	15/15	Recurrence rate: 5/15 with IFN- α 12/15 without treatment	[142]
2002	Advanced HCC: GM-CSF + IFN- γ	15	OS after 26 weeks: 40%	[143]
	Advanced HCC: low dose IL-2	18	2/18 CR 1/18 PR	[144]
	Advanced HCC: dendritic cells pulsed with autologous tumor	2	Feasibility	[145]
2003	Advanced HCC: dendritic cells pulsed with autologous tumor	10	Feasibility	[97]
	Advanced HCC: 5-FU IFN- α 2b	43	1/36 CR 8/36 PR	[146]
	Advanced HCC: dendritic cells pulsed with autologous tumor	1	Feasibility	[147]
	Advanced HCC: doxorubicin + 5-FU IFN- α 2b	30	2/30 PR	[148]
	Advanced HCC: cisplatin + 5-FU IFN- α	6	2/6 PR	[149]
	Advanced HCC: AFP peptide vaccination	6	All patients developed T cell responses	[100]
2004	Adjuvant: formalin-fixed tumor vaccine vs. no treatment	9/13	Recurrence rate: 3/18 with vaccine 13/21 without treatment	[99]
	Advanced HCC: cytokine induced killer cells	13	Feasibility	[89]
	Advanced HCC: intratumoral adenovirus encoding IL-12	9	1/9 PR	[150]
	Advanced HCC: 131I-Hepama-1 mAb	32	Median survival: 3 months	[151]
2005	Advanced HCC:	31	4/31 PR	[98]
	Advanced HCC: intratumoral dendritic cell injection	4	Feasibility	[94]
	Advanced HCC: cisplatin + doxorubicin + 5-FU + IFN-2 α	26	4/26 PR	[152]
	Advanced HCC: local radiation + intratumoral DC injection	14	2/12 PR	[95]
2006	Advances HCC: dendritic cells pulsed with AFP peptides	10	AFP-specific T cells in 6/10 patients	[91]

RFTA (Radiofrequency thermal ablation) demonstrated an increase in the frequency of tumor reactive circulating T cells [101]. In this study, IFN- γ secretion by CD8⁺ T cells was tested before and 4 weeks after treatment, upon stimulation with autologous tumor lysate. Ablation therapy not only increased the number of tumor-specific T cells circulating in peripheral blood, but also the frequency of T cells specific for recall antigens. No correlation between T cell responses and protection from tumor relapse was found. Although this study was not primarily designed to show an effect of RFTA on T cell responses, results from this study suggest that RFTA can activate non-specific immune responses of T lymphocytes. Another study has evaluated the effect of RFTA or PEI on the function of dendritic cells in eight patients *in vivo* [102]. Local ablative therapy resulted in decreased numbers of DCs 7 and 14 days after treatment in parallel with an increase of CD83 expression.

Although local ablative therapies as well as surgery has been shown to be very effective for the treatment of HCC, recurrence rates are still high [103] due to a high risk of metastasis and development of *de novo* HCC in a cirrhotic liver. Therefore, effective adjuvant therapy has to not only effectively prevent the development of tumor metastasis, but should also stop the development of *de novo* tumors in patients with liver cirrhosis [104].

10. Outlook

The efficacy of anti-tumor vaccines has been proven in a variety of animal models. However, the bigger challenge lies in the transition from experimental models to clinical trials. HCC is a difficult tumor to treat and most current therapeutic options have only limited success. As for immunotherapeutic approaches to HCC, the identification of new antigens as well as modulation of immune response, reversal of tolerance and removal of inhibitory factors all need to be considered. Since tumor antigens are mainly self-antigens, an effective immunotherapeutic approach should reverse the tolerant state of the immune system (to the antigen) and activate low avidity T cells. Future immunotherapy trials should be randomized, include high enough patient numbers and a thorough immune monitoring should become mandatory. Only the combination of well-designed clinical trials with innovative immunotherapeutic approaches will lead to the development of efficient new therapies for the treatment of HCC.

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