T Cell-Based Therapies of HCC

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The scope of therapeutic options for the treatment of hepatocellular carcinoma (HCC) has recently been expanded by immunotherapeutic regimens. T cell-based therapies, especially in combination with other treatments have achieved far better outcomes compared to conventional treatments alone. However, there is an emerging body of evidence that eliciting T cell responses in immunotherapeutic approaches is insufficient for favorable outcomes.

Keywords: immune checkpoint inhibition ; tumor surveillance ; T cell responses ; treatment failure ; CAR therapy ; HBV ; HCV

1. Introduction

Patients with advanced stages of HCC face a poor prognosis. Liver cancer is the third leading cause of cancer-related mortality worldwide [1]. It is still a difficult-to-treat disease, despite several treatment options, such as liver transplantation, systemic treatment with chemotherapy, loco-regional treatment, such as transarterial chemoembolization (TACE) and radioembolization or treatment with sorafenib, lenvatinib, or other multi-kinase inhibitors [2][3][4]. Untreated HCC has a 5-year overall survival rate of less than 10% and curative options in advanced stages, when the disease is usually detected, are rare [5]. Recently, prognosis of HCC had improved remarkably with the implementation of immune checkpoint inhibition (CPI) into the treatment schemes as we will discuss in detail. The agents that are commonly used for CPI are antibodies inhibiting the CTLA4 pathway, such as ipilimumab, and the PD-1/PD-L1 pathway, such as pembrolizumab and atezolizumab. CTLA-4 is a homologue of CD28 that binds to members of the B7 family during T cell activation by antigen-presenting cells and has a higher affinity than does CD28. The interaction of PD-1 with PD-L1 keeps T cells from killing tumor cells, whereas blocking this interaction can allow for cytotoxic responses to lyse tumor cells.

Not even a decade has passed from the time when concrete evidence was found that lymphocytes can prevent tumor development [6] until the first clinical studies of checkpoint inhibition confirmed increased survival in patients [7][8]. In 2010, patients with metastatic melanoma benefitted from treatment with ipilimumab, an inhibitor of the CTLA-4 pathway. PD-1/PD-L1 checkpoint inhibitors soon followed suit, also showing effectiveness of checkpoint inhibition in other tumor entities [9][10]. It was realized that CPI elicits T cells against cancer neoantigens as the main drivers of responses [11][12]. Since occurrence of positive clinical studies, a plethora of checkpoint inhibitors targeting PD-1 and PD-L1 has been approved and tested in clinical trials in a great variety of cancers. The results of phase 1/2 and 2 clinical trials in HCC patients with single use of blocking antibodies of the PD-1 pathway led to approval of these agents by the United States Food and Drug Administration (FDA) for the treatment of HCC [13][14]. However, the first phase III trials failed to reach the predefined endpoint both for nivolumab as a first line therapy and pembrolizumab (both inhibitors of the PD-1 pathway) as second line treatment [15][16]. Still, both trials confirmed an overall response rates (ORR) of 15–20% observed in the phase II trials. However, among these responding patients, complete responses defined as disappearance of vital tumors were almost non-existent.

2. Biomarkers and Immunological Classification of HCC

Predicting therapeutic benefit prior to or shortly after therapy starts by biomarkers is a well sought-after aim in clinical oncology [17]. So far, the most common marker that is correlated to a therapeutic response in other tumor entities is the expression of PD-L1 in tumor-tissue and the tumor mutational burden (TMB). However, these parameters have not been shown to reliably predict treatment responses in HCC patients receiving checkpoint inhibitors [18][19][20]. One study even questions the dominant role of neoantigens in HCC for CPI due to the relatively low mutational burden compared to malignant melanoma [21]. The lack of common markers for the prediction of the treatment response of HCC led to other assessments as we will see later.

Anti-tumor immunity can appear concomitantly with tumor progression. This observation is called the “Hellström paradox” according to a study from 1968 that found humoral and cellular components with tumoricidal activity in patients with growing tumors [22]. These findings suggests that activity of tumor-directed immunity must outpace tumor cell proliferation to reach a threshold of net reduction in the overall tumor mass. This view conforms also with the state of equilibrium derived from the hypothesis of immunoediting [23]. Accordingly, cancer immunotherapy aims at amplification of existing tumor immunity or de novo generation in order to tip the scale towards favourable outcomes [24]. With regard to HCC, the stratification of etiologies for the clinical outcome may help to dissect and understand effects of signaling pathways and immune cell phenotypes on immunotherapy responses [25]. It is important to distinguish the non-responsiveness to cancer immunotherapy between the failure of triggering an immune response and the functional failure of the elicited response. Here, primary, adaptive, and acquired resistance can be differentiated [26]. Acquired resistance is an important, but often underestimated, clinical parameter showing that responses are mostly temporary. Thus, the design of future clinical studies should include strategies to maintain already existing immune responses. The overall designation of all immune factors within a host that eventually leads to the killing of cancer cells is briefly called the cancer immune cycle [27]. Any interruption within that sequence or the functionality of essential networks of the cycle leads to a complete abortion and eventual failure of tumor rejection. It is a convenient tenet and the ultimate reason to explain resistance to cancer immunotherapy and treatment failure of CPI. A more refined view on the clinical effects of checkpoint inhibitors is the cancer immune set point. This views immunity to cancer as a complex set of tumor, host and environmental factors. These factors govern the magnitude and timing of the anticancer response [28]. Consistent with the observation that HCC develops in a complex environment of chronic hepatitis and fibrosis, likewise the genomic landscape has been described as highly complex and heterogeneous [29][30]. More suitable for predictions appear multi-omics approaches that have been proposed for immune profiling of HCC. A study of Sia et al. analysed 956 HCC samples and found that about 25% of HCC have markers of an inflammatory response with high expression levels of PD-1 and PD-L1, cytotoxic marker expression, such as an interferon gamma (IFN-γ) signature, and low levels of chromosomal aberrations. The immune class correlated with better overall survival [31]. Additionally, the subgroup of this cohort was either characterized by an adaptive T cell response or an exhausted immune response that allowed stratification of an active and exhausted immune subclass. The active immune subclass showed signs of an ongoing cytotoxic response, in which IFN-γ and granzyme B signatures are present. In contrast, the immune-excluded subclass was dominated by signature of T cell exhaustion, suppressive myeloid cells, and tumor growth factor-β (TGF-β). In another study, Zhang et al. performed immune profiling of HCC and defined three groups that suggest differentiation into immunocompetent, immunosuppressive, and immunodeficient subtypes [32]. Expression level analysis of CD45 and Foxp3 in immunohistochemistry (IHC) allowed for correlative classification of the treatment outcome in this study. The immunocompetent subtype was CD45 hi and FOXP3 lo showing infiltration of αβ and γδ T cells. Furthermore, HCCs of the immunosuppressive subtype stained CD45 hi and FOXP3 hi indicating regulatory T and B cells, as well as tolerogenic macrophages and immunosuppressive molecules, such as PD-1/PD-L1, TGF-β, VEGF, T cell immunoglobulin and mucin domain containing protein 3 (TIM-3) and interleukin-10 (IL-10). The immunodeserted subtype showed a CD45 lo phenotype with a significant reduction of immune cell infiltration [32]. A similar classification of the immune composition of HCC by a study investigating 158 HCC patients was proposed to distinguish three immune-subtypes: Immune-high, immune-mid, and immune-low. Increased plasma/B cell and T cell infiltration in the immune-high subtype were identified as independent positive prognostic factors [33]. These promising studies show that an in-depth immune profiling potentially combined with genetic approaches may lead to stratification of HCC for appropriate prediction of the outcome. In addition, these studies suggest that T cells with distinct properties exists that prevent tumor outgrowth.

3. HCC Immune Surveillance by T Cells

The immune surveillance of the liver is a well-studied topic that has revealed several mechanisms throughout different stages of liver cancer development for protection of the host (reviewed in [34]). During the pre-malignant phase of tumor development it has been shown that senescence surveillance is the driving force for the elimination of pre-cancerous and senescent hepatocytes with a secretory phenotype by CD4 T cells and macrophages [35]. Upon progression to the malignant phase, nascent tumor cells are primarily under control of CD4 and CD8 T cells [23]. T cell responses directed against tumor-associated antigens (TAA) in HCC patients are frequently observed and the presence of responses are correlated with survival [36][37][38]. Strong T cell responses directed against TAA are also correlated with suppression of recurring HCC after therapeutic regimens [39]. Well described TAA-responses are directed against alpha-fetoprotein (AFP), human telomerase reverse transcriptase (hTERT), glypican-3 (GPC3), melanoma-associated gene-A (MAGE-A), squamous cell carcinoma antigen recognized by T cells (SART), and New York-esophageal squamous cell carcinoma-1 (NY-ESO-1) [40][41][42][43][44][45].

The importance of HCC immune surveillance by T cells using TAAs has been investigated in patients with liver cirrhosis upon HCV clearance by antiviral therapies [46]. Cirrhotic patients had an increased frequency of CD4 and CD8 T cells that secreted IFN-γ after stimulation with GPC3 peptide pools. Moreover, those patients who developed HCC after antiviral therapy had CD4 and CD8 T cells with significantly lower cytokine release and proliferative capacity compared to those patients that remained tumor-free. Higher magnitudes of GPC3 reactive T cells also delayed diagnosis of HCC developers according to the time of HCC emergence after initiation of antiviral therapy. This study clearly shows the link between the importance of tumor-specific T cells not only in relation to delayed HCC onset, but also for the relevance of immune surveillance for preventing liver cancer [46]. The crucial role of T cells for anti-tumor surveillance has also been demonstrated in a mouse model of liver cancer. Liver tumors were established by transposon-mediated gene transfer. Transposons coding for oncogenic ras linked to potent CD4 and CD8 T cell epitopes was used to transform hepatocytes into nascent tumors with tailored tumor immunogenicity. Potent T cell responses and tumor growth suppression was detected when both, CD4 and CD8 T cell epitopes were expressed. A lack of CD4 tumor-specific epitopes led to induction of robust amounts of tumor-specific CD8 T cells that were incapable of tumor surveillance. On the other hand, presence of CD4 tumor-specific epitopes combined with a lack of CD8 tumor-specific epitopes neither led to CD4, nor to CD8 T cell responses, showing the mutual dependence that is necessary for efficient liver cancer immune surveillance [47].

Although HCC immune surveillance can be regarded as a pivotal mechanism in terms of tumor development, progression, and prognosis, a recent seminal study demonstrated its limitations in non-alcoholic steatohepatitis (NASH), which is an important driver of HCC. The authors observed in preclinical models of NASH-induced HCC that CPI treatment expanded activated PD-1 + CD8 T cells but did not lead to tumor remission. Single cell sequencing of cells expressing T cell receptor β (TCRβ) showed gene expression profiles of cytotoxicity and effector-functions together with elevated traits of exhaustion, i.e., Pdcd1 and Tox . PD-1 + CD8 T cells accumulated to high numbers of NASH-HCC mice in the liver with a resident-like T cell character. At a first glance, it may appear counterintuitive that accumulation of CD8 T cells within tumor-tissue, that is usually associated with a good prognosis, leads to a failure of immunotherapies in NASH-HCC. However, depletion of CD8 T cells in this model with a preventive setup provided a significant protection from liver damage and HCC development, suggesting that liver CD8 T cells actively promote HCC in NASH. Moreover, the study found similar results in patients. PD-1 + CD8 T cells with a residency phenotype were found in two independent NASH cohorts. Interestingly, the magnitude of hepatic PD-1 + CD8 T cells directly correlated with body-mass index and the extent of liver damage. Single cell RNA-seq revealed similar gene expression signatures that were also found in mice, i.e., PDCD1 , GZMB , TOX , CXCR6 , RGS1 , and SELL . Furthermore, a meta-analysis of three large randomized controlled phase III trials of immunotherapies in patients with advanced HCC, namely Checkmate-495, IMbrave150, and Keynote-240 [15][16][48], showed that anti-PD-1 or anti-PD-L1 treatment in the control arm led to superior outcome in patients with HBV- and HCV-related HCC, but not in patients with non-viral HCC. However, this meta-analysis did not differentiate between different lines of treatment and between alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD) or NASH. Further investigation revealed that NAFLD was independently associated with shortened survival of patients with HCC after CPI. Hence, this study provides a rationale for stratification of HCC patients according to their etiology of cancer [49]. In line with these results, Heinrich et al. studied the effect of immunotherapy on tumors in the liver in the context of steatohepatitis. Here, application of M30-RNA vaccine or an anti-OX40 antibody led to growth inhibition of intrahepatic B16 melanoma and CT26 colon cancer cells without steatohepatitis. In the same experimental setup with additional diet-induced steatohepatitis, however, immunotherapy led to progressive tumor growth and a loss of CD4 T cells from the liver. The application of reactive oxygen species (ROS)-reducing N-acetylcysteine rescued the amount of intratumor CD4 T cells in mice with steatohepatitis and recovered therapeutic efficacy [50]. These results suggest an in situ mechanism of NASH with regard to failure on immunotherapies and furthermore identifies a putative strategy to overcome detrimental effects of NASH on CD4 T cell tumor immunity by protecting these cells from ROS-mediated damage. It will be intriguing to see whether the application of N-acetylcysteine is sufficient to restore tumor immunity in NASH-HCC patients and if this may even prevent NASH patients from CD8 T cell mediated liver damage and subsequent tumor development by reintroducing proper CD4-mediated regulation of CD8 T cell responses [49][50].

4. Other Immunotherapeutic Approaches of HCC

The liver being an exceptional organ when it comes to tolerance induction, this organ is mediating the ‘liver tolerance effect’ with regard to local and systemic tolerance to self and foreign antigens [51]. Liver cancer may exploit multiple mechanisms of this effect to ward off or silence tumor immunity. As already mentioned, senescence surveillance limits the outgrowth of pre-malignant hepatocytes [35]. However, if senescent cells are not cleared, they may give rise to HCCs that block maturation of CCR2 + myeloid cells. This cell type is required to execute the senescence program, and ablation of CCR2 leads to development of HCC. Inhibiting the maturation of myeloid precursors leads in turn to inhibition of NK cell functions and exacerbates HCC progression. Hence, the secretory phenotype of senescent hepatocytes leads to

suppression of liver cancer in early stages of tumor development, but they may accelerate tumor progression in the late stages of HCC. It appears promising to investigate immunotherapies combining multiple strategies that include blocking the CCL2/CCR2 axis thereby enhancing NK cell infiltration and activity [52]. Loco-regional treatments in HCC are known to stimulate tumor immunity due to massive release of antigens from dying tumor cells. This may synergize with CPI and other immunotherapies. One study sought to trigger CD8 T cell immunity by ablative methods and used CPI to further stimulate T cell immunity. Ablation was performed by a TACE or radiofrequency ablation (RFA) combined with tremelimumab, a CTLA-4 inhibitor. The authors established this approach as a putative new treatment approach that leads to the accumulation of CD8 T cells with a correlation of a positive clinical activity [53]. Similarly, the combination of RFA with a dendritic cell vaccine based on monocyte-derived DCs stimulated with OK432 was well tolerated. This treatment combination improved TAA-specific T cell responses and the 5-year recurrence-free survival was significantly higher with 50% in the combined treatment group compared to 7.7% in patients without combined treatment [54]. Other clinical studies for HCC, e.g., IMMUTACE (TACE combined with nivolumab, NCT03572582), IMMULAB (RFA combined with pembrolizumab, NCT03753659), or IMMUWIN (selective internal radiation therapy (SIRT) combined with durvalumab (antibody specific for PD-L1), NCT04522544) are currently active to fathom loco-regional approaches with CPI. These and other studies (reviewed in [55]) will reveal synergies between established clinical treatment options with immunotherapies to improve the outcome for HCC patients.

Oncolytic virotherapy (OV) is a promising approach for the treatment of solid tumors. Viral vectors can be genetically modified to replicate in primarily in tumor tissue [56]. In pre-clinical models OV has shown promising results in combination with checkpoint inhibitors [57]. Mechanistically, OV appears to broaden the spectrum of tumor-directed T cell responses when combined with CPI. Viral replication in tumors induces expression of PD-1 on metastasis and inhibits dissemination, if mice were treated with PD-1 blocking antibodies in a liver cancer model [58]. In clinical settings, the oncolytic vector talimogene laherparepvec (T-vec), a herpes simplex virus type-1 armed with an expression cassette of granulocyte macrophage colony-stimulating factor (GM-CSF) to enhance antitumor immunity, has been used to treat patients with advanced melanoma in a phase III study [59]. This clinical study published in 2015 was the first phase III study with OV that led to approval of the FDA. Clinical studies investigating OV for the treatment of liver cancer have also been performed. The oncolytic and immunotherapeutic virus JX-549 (Pexastimogene devacirepvec or Pexa-Vec) based on a vaccinia virus also expresses GM-CSF and was evaluated in a randomized phase I/II dose-finding study. Pexa-Vec was well tolerated and showed tumor responses and dose-related survival in individuals with HCC [60]. In a subsequent phase IIb study, Pexa-Vec did not improve the overall survival of HCC patients as a second line treatment after a sorafenib failure. It was furthermore postulated that virotherapy has more potential in earlier disease stages [61]. At that time, pre-clinical studies appear particularly incongruent in comparison to clinical studies with regard to therapeutic efficiency of oncolytic virotherapy. However, first clinical studies of OV and CPI have been already performed in melanoma, in part with promising outcomes [62][63] and now there is also a combinatorial first line phase I/IIa study of oncolytic virotherapy (Pexa-Vec) with nivolumab in HCC patients ongoing (NCT03071094). Also other tumor entities such as glioblastoma show promising results for safety and efficacy in recent clinical trials with OV [64]. In light of these results and the probable high potency of OV especially in combination with CPI, new clinical trials should be encouraged to further improve the prognosis and therapeutic options for HCC.

Adoptive transfer of autologous lymphocytes derived from tumor tissue against overexpressed self-derived differentiation antigens has shown promising results in a subgroup of melanoma patients almost two decades ago [65]. The transferred cells were proliferating in vivo after ex vivo expansion, displayed functional activity, and were able to traffic to tumors. This proof-of-concept study invigorated a new therapeutic field of adoptive cell therapy (ACT). Since then, ACT of chimeric antigen receptor- (CAR-) T cells such as lisocabtagene maraleucel for refractory B cell lymphoma induced durable responses and a manageable long-term safety profile [66][67]. However, CAR T-cells can mediate severe adverse effects. Treated patients must be monitored closely for cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome [68]. ACT comprises cells that mediates cellular immunity, such as CD8 T cells, iNKT cells (invariant NK T cells), γδ T cells, cytokine-induced immune killer cells, and CAR-T cells. Several clinical studies with ACT are being conducted. For example, a phase I/II study uses iNKT cells and PD-1 + CD8 T cells, that are assumed to be tumor specific, are used to treat various cancers, including HCC (NCT03093688). Other clinical studies use highly purified CTLs (cytotoxic lymphocytes) in combination with RFA (NCT02678013) or resection (NCT02709070) that have already reached primary completion. With regard to ACT of CAR-T cells, pre-clinical studies of patient-derived xenografts or orthotopic liver cancer, ACT of anti-GPC3 CAR-T cells have delivered positive results [69][70]. There are clinical studies ongoing (NCT04121273, NCT02905188, NCT03198546) that use GPC3 CAR-T cells. It has been shown that >70% of HCCs are positive for GPC3 and GPC3 expression is correlated with a poor prognosis [71]. Shi et al. published results from a first phase I CAR-GPC3 T cell study in 13 patients and found early signs of anti-tumor activity of these cells in HCC. The described safety profile included 9 patients with cytokine release syndrome [72]. One phase I study, that is applicable to HLA-A2 + patients, utilizes autologous genetically modified AFP c332 T cells for the treatment of HCC

(NCT03132792). First promising results have already been presented (overview for this and other ACT/HCC studies in [73]). In this clinical study, targeting AFP + HCC tumors with AFP-specific CAR-T cells resulted in one complete response out of four patients and one patient had a partial response with 100% reduction of targeted tumors and only one non-targeted tumor nodule remained at therapy week eight. The application of CAR-T cells targeting a single antigen is likely to underlie immune escape and thus leading to treatment failure, especially when non-essential antigens for tumor survival are selected [23][74]. Hence, selection of multiple targets may lead to a higher success rate. For instance, study NCT03638206 impeded this putative pitfall and selected DR-5, C-met, and EGFR V III as CAR-T cell targets for the treatment of HCC. A comprehensive review including a list of clinical studies for HCC can be found elsewhere [75].

As one of the first vaccination approaches in HCC therapy, peptide immunizations have been employed to generate de novo cancer-specific T cell responses. Initial vaccination studies primarily focused on AFP, an oncofetal target which is expressed in approximately 50% of all HCCs. While the initial studies with AFP peptide-pulsed dendritic cells showed limited therapeutic efficacy [76], a more recent trial with AFP peptides emulsified in incomplete Freund's adjuvant demonstrated clinical efficacy with one complete response and several patients with long-term disease control without severe side effects [77]. Since increased telomerase expression due to telomerase promoter mutations is a hallmark of HCC, vaccines targeting the catalytic telomerase subunit hTERT have been employed in a number of clinical trials. As an example, the peptide vaccine GV1001 targeting the hTERT epitope 611-626 was tested in a phase 2 trial in combination with GM-CSF and cyclophosphamide [78]. While the vaccinations were well-tolerated, no clear telomerase-specific T cells were detected, and clinical responses were limited. In another phase I study, HLA-A24 specific hTERT-specific peptides were used for adjuvant HCC treatment following radiofrequency ablation [79]. Side effects were mostly transient and limited to the skin while a trend towards lower cancer recurrence was noted in patients with detectable hTERT-specific immune responses. As a third prominent target, GPC3 has been subject of both preclinical and clinical trials due to its convincing specificity for HCC and its role as a negative prognostic factor. In an early phase I trial, intradermal peptide injection induced a partial response in one patient and a correlation between GPC3-specific immune responses and overall survival was noted [80]. Similar results were obtained in another phase I study in patients with advanced HCC with one partial response and several patients reaching stable disease [81]. These clinical trials highlight the potential of vaccination studies in HCC but reveal yet unsolved limitations regarding the quantity and quality of cancer-specific T cells induced by current vaccination regimens.

References

1. Forner, A.; Reig, M.; Bruix, J. Hepatocellular carcinoma. Lancet 2018, 391, 1301–1314.
2. Crocetti, L.; Bargellini, I.; Cioni, R. Loco-regional treatment of HCC: Current status. Clin. Radiol. 2017, 72, 626–635.
3. Cheng, A.-L.; Kang, Y.-K.; Chen, Z.; Tsao, C.-J.; Qin, S.; Kim, J.S.; Luo, R.; Feng, J.; Ye, S.; Yang, T.-S.; et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009, 10, 25–34.
4. Chen, Z.H.; Hong, Y.F.; Chen, X.; Chen, J.; Lin, Q.; Lin, J.; Li, X.; Wen, J.Y.; Ruan, D.Y.; Dong, M.; et al. Comparison of five staging systems in predicting the survival rate of patients with hepatocellular carcinoma undergoing trans-arterial chemoembolization therapy. Oncol. Lett. 2018, 15, 855–862.
5. Giannini, E.G.; Farinati, F.; Ciccarese, F.; Pecorelli, A.; Rapaccini, G.L.; Di Marco, M.; Benvegnù, L.; Caturelli, E.; Zoli, M.; Borzio, F.; et al. Prognosis of untreated hepatocellular carcinoma. Hepatology 2015, 61, 184–190.
6. Shankaran, V.; Ikeda, H.; Bruce, A.T.; White, J.M.; Swanson, P.E.; Old, L.J.; Schreiber, R.D. IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001, 410, 1107–1111.
7. Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C.; et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N. Engl. J. Med. 2010, 363, 711–723.
8. Maker, A.V.; Phan, G.Q.; Attia, P.; Yang, J.C.; Sherry, R.M.; Topalian, S.L.; Kammula, U.S.; Royal, R.E.; Haworth, L.R.; Levy, C.; et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: A phase I/II study. Ann. Surg. Oncol. 2005, 12, 1005–1016.
9. Brahmer, J.R.; Tykodi, S.S.; Chow, L.Q.M.; Hwu, W.-J.; Topalian, S.L.; Hwu, P.; Drake, C.G.; Camacho, L.H.; Kauh, J.; Odunsi, K.; et al. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. N. Engl. J. Med. 2012, 366, 2455–2465.
10. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B.; et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. N. Engl. J.

Med. 2012, 366, 2443–2454.

11. Van Rooij, N.; Van Buuren, M.M.; Philips, D.; Velds, A.; Toebes, M.; Heemskerk, B.; Van Dijk, L.J.; Behjati, S.; Hilkmann, H.; El Atmioui, D.; et al. Tumor Exome Analysis Reveals Neoantigen-Specific T-Cell Reactivity in an Ipilimumab-Responsive Melanoma. J. Clin. Oncol. 2013, 31, e439–e442.
12. Gubin, M.M.; Zhang, X.; Schuster, H.; Caron, E.; Ward, J.P.; Noguchi, T.; Ivanova, Y.; Hundal, J.; Arthur, C.D.; Krebber, W.J.; et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014, 515, 577–581.
13. El-Khoueiry, A.B.; Sangro, B.; Yau, T.C.C.; Crocenzi, T.S.; Kudo, M.; Hsu, C.; Kim, T.-Y.; Choo, S.-P.; Trojan, J.; Welling, T.H.; et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 2017, 389, 2492–2502.
14. Zhu, A.X.; Finn, R.S.; Edeline, J.; Cattan, S.; Ogasawara, S.; Palmer, D.; Verslype, C.; Zagonel, V.; Fartoux, L.; Vogel, A.; et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): A non-randomised, open-label phase 2 trial. Lancet Oncol. 2018, 19, 940–952.
15. Finn, R.S.; Ryoo, B.-Y.; Merle, P.; Kudo, M.; Bouattour, M.; Lim, H.Y.; Breder, V.; Edeline, J.; Chao, Y.; Ogasawara, S.; et al. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. J. Clin. Oncol. 2020, 38, 193–202.
16. Yau, T.; Park, J.W.; Finn, R.S.; Cheng, A.L.; Mathurin, P.; Edeline, J.; Kudo, M.; Han, K.H.; Harding, J.J.; Merle, P.; et al. CheckMate 459: A randomized, multi-center phase III study of nivolumab (NIVO) vs sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). Ann. Oncol. 2019, 30, v874–v875.
17. Gnjatic, S.; Bronte, V.; Brunet, L.R.; Butler, M.O.; Disis, M.L.; Galon, J.; Hakansson, L.G.; Hanks, B.A.; Karanikas, V.; Khleif, S.N.; et al. Identifying baseline immune-related biomarkers to predict clinical outcome of immunotherapy. J. Immunother. Cancer 2017, 5, 44.
18. Taube, J.M.; Klein, A.; Brahmer, J.R.; Xu, H.; Pan, X.; Kim, J.H.; Chen, L.; Pardoll, D.M.; Topalian, S.L.; Anders, R.A. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti–PD-1 Therapy. Clin. Cancer Res. 2014, 20, 5064–5074.
19. Nabet, B.Y.; Esfahani, M.S.; Moding, E.J.; Hamilton, E.G.; Chabon, J.J.; Rizvi, H.; Steen, C.B.; Chaudhuri, A.A.; Liu, C.L.; Hui, A.B.; et al. Noninvasive Early Identification of Therapeutic Benefit from Immune Checkpoint Inhibition. Cell 2020, 183, 363–376.e13.
20. Ang, C.; Klempner, S.J.; Ali, S.M.; Madison, R.; Ross, J.S.; Severson, E.A.; Fabrizio, D.; Goodman, A.; Kurzrock, R.; Suh, J.; et al. Prevalence of established and emerging biomarkers of immune checkpoint inhibitor response in advanced hepatocellular carcinoma. Oncotarget 2019, 10, 4018–4025.
21. Löffler, M.W.; Mohr, C.; Bichmann, L.; Freudenmann, L.K.; Walzer, M.; Schroeder, C.M.; Trautwein, N.; Hilke, F.J.; Zinser, R.S.; Muhlenbruch, L.; et al. Multi-omics discovery of exome-derived neoantigens in hepatocellular carcinoma. Genome Med. 2019, 11, 28.
22. Hellström, I.; Hellström, K.E.; Pierce, G.E.; Yang, J.P.S. Cellular and Humoral immunity to Different Types of Human Neoplasms. Nature 1968, 220, 1352–1354.
23. Dunn, G.P.; Old, L.J.; Schreiber, R.D. The Three Es of Cancer Immunoediting. Annu. Rev. Immunol. 2004, 22, 329–360.
24. Gao, Q.; Qiu, S.-J.; Fan, J.; Zhou, J.; Wang, X.-Y.; Xiao, Y.-S.; Xu, Y.; Li, Y.-W.; Tang, Z.-Y. Intratumoral Balance of Regulatory and Cytotoxic T Cells Is Associated With Prognosis of Hepatocellular Carcinoma After Resection. J. Clin. Oncol. 2007, 25, 2586–2593.
25. Pinter, M.; Scheiner, B.; Peck-Radosavljevic, M. Immunotherapy for advanced hepatocellular carcinoma: A focus on special subgroups. Gut 2021, 70, 204–214.
26. Sharma, P.; Hu-Lieskovan, S.; Wargo, J.A.; Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell 2017, 168, 707–723.
27. Chen, D.S.; Mellman, I. Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity 2013, 39, 1–10.
28. Chen, D.S.; Mellman, I. Elements of cancer immunity and the cancer–immune set point. Nature 2017, 541, 321–330.
29. Adeniji, N.; Dhanasekaran, R. Genomic Landscape of HCC. Curr. Hepatol. Rep. 2020, 19, 448–461.
30. Li, S.; Mao, M. Next generation sequencing reveals genetic landscape of hepatocellular carcinomas. Cancer Lett. 2013, 340, 247–253.
31. Sia, D.; Jiao, Y.; Martinez-Quetglas, I.; Kuchuk, O.; Villacorta-Martin, C.; de Moura, M.C.; Putra, J.; Campreciós, G.; Bassaganyas, L.; Akers, N.; et al. Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on

Molecular Features. Gastroenterology 2017, 153, 812–826.

32. Zhang, Q.; Lou, Y.; Yang, J.; Wang, J.; Feng, J.; Zhao, Y.; Wang, L.; Huang, X.; Fu, Q.; Ye, M.; et al. Integrated multiomic analysis reveals comprehensive tumour heterogeneity and novel immunophenotypic classification in hepatocellular carcinomas. Gut 2019, 68, 2019–2031.
33. Kurebayashi, Y.; Ojima, H.; Tsujikawa, H.; Kubota, N.; Maehara, J.; Abe, Y.; Kitago, M.; Shinoda, M.; Kitagawa, Y.; Sakamoto, M. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. Hepatology 2018, 68, 1025–1041.
34. Ostroumov, D.; Fekete-Drimusz, N.; Saborowski, M.; Kühnel, F.; Woller, N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. Cell. Mol. Life Sci. 2018, 75, 689–713.
35. Kang, T.-W.; Yevsa, T.; Woller, N.; Hoenicke, L.; Wuestefeld, T.; Dauch, D.; Hohmeyer, A.; Gereke, M.; Rudalska, R.; Potapova, A.; et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature 2011, 479, 547–551.
36. Liang, J.; Ding, T.; Guo, Z.-W.; Yu, X.-J.; Hu, Y.-Z.; Zheng, L.; Xu, J. Expression pattern of tumour-associated antigens in hepatocellular carcinoma: Association with immune infiltration and disease progression. Br. J. Cancer 2013, 109, 1031–1039.
37. Flecken, T.; Schmidt, N.; Hild, S.; Gostick, E.; Drognitz, O.; Zeiser, R.; Schemmer, P.; Bruns, H.; Eiermann, T.; Price, D.A.; et al. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. Hepatology 2014, 59, 1415–1426.
38. Hofmann, M.; Tauber, C.; Hensel, N.; Thimme, R. CD8+ T Cell Responses during HCV Infection and HCC. J. Clin. Med. 2021, 10, 991.
39. Hiroishi, K.; Eguchi, J.; Baba, T.; Shimazaki, T.; Ishii, S.; Hiraide, A.; Sakaki, M.; Doi, H.; Uozumi, S.; Omori, R.; et al. Strong CD8+ T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. J. Gastroenterol. 2010, 45, 451–458.
40. Boon, T.; Van Der Bruggen, P. Human tumor antigens recognized by T lymphocytes. J. Exp. Med. 1996, 183, 725–729.
41. Rosenberg, S.A. A New Era for Cancer Immunotherapy Based on the Genes that Encode Cancer Antigens. Immunity 1999, 10, 281–287.
42. Scanlan, M.J.; Gure, A.O.; Jungbluth, A.A.; Old, L.J.; Chen, Y.-T. Cancer/testis antigens: An expanding family of targets for cancer immunotherapy. Immunol. Rev. 2002, 188, 22–32.
43. Mizukoshi, E.; Nakamoto, Y.; Marukawa, Y.; Arai, K.; Yamashita, T.; Tsuji, H.; Kuzushima, K.; Takiguchi, M.; Kaneko, S. Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma. Hepatology 2006, 43, 1284–1294.
44. Kaji, K.; Mizukoshi, E.; Yamashita, T.; Arai, K.; Sunagozaka, H.; Fushimi, K.; Nakagawa, H.; Yamada, K.; Terashima, T.; Kitahara, M.; et al. Cellular Immune Responses for Squamous Cell Carcinoma Antigen Recognized by T Cells 3 in Patients with Hepatocellular Carcinoma. PLoS ONE 2017, 12, e0170291.
45. Thimme, R.; Neagu, M.; Boettler, T.; Neumann-Haefelin, C.; Kersting, N.; Geissler, M.; Makowiec, F.; Obermaier, R.; Hopt, U.T.; Blum, H.E.; et al. Comprehensive analysis of the α-fetoprotein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. Hepatology 2008, 48, 1821–1833.
46. Sekyere, S.O.; Schlevogt, B.; Mettke, F.; Kabbani, M.; Deterding, K.; Wirth, T.C.; Vogel, A.; Manns, M.P.; Falk, C.S.; Cornberg, M.; et al. HCC Immune Surveillance and Antiviral Therapy of Hepatitis C Virus Infection. Liver Cancer 2019, 8, 41–65.
47. Knocke, S.; Fleischmann-Mundt, B.; Saborowski, M.; Manns, M.P.; Kühnel, F.; Wirth, T.C.; Woller, N. Tailored Tumor Immunogenicity Reveals Regulation of CD4 and CD8 T Cell Responses against Cancer. Cell Rep. 2016, 17, 2234–2246.
48. Finn, R.S.; Qin, S.; Ikeda, M.; Galle, P.R.; Ducreux, M.; Kim, T.-Y.; Kudo, M.; Breder, V.; Merle, P.; Kaseb, A.O.; et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N. Engl. J. Med. 2020, 382, 1894–1905.
49. Pfister, D.; Núñez, N.G.; Pinyol, R.; Govaere, O.; Pinter, M.; Szydlowska, M.; Gupta, R.; Qiu, M.; Deczkowska, A.; Weiner, A.; et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021, 592, 450–456.
50. Heinrich, B.; Brown, Z.J.; Diggs, L.P.; Vormehr, M.; Ma, C.; Subramanyam, V.; Rosato, U.; Ruf, B.; Walz, J.S.; McVey, J.C.; et al. Steatohepatitis Impairs T-cell–Directed Immunotherapies Against Liver Tumors in Mice. Gastroenterology 2021, 160, 331–345.e6.
51. Tiegs, G.; Lohse, A.W. Immune tolerance: What is unique about the liver. J. Autoimmun. 2010, 34, 1–6.

52. Eggert, T.; Wolter, K.; Ji, J.; Ma, C.; Yevsa, T.; Klotz, S.; Medina-Echeverz, J.; Longerich, T.; Forgues, M.; Reisinger, F.; et al. Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. Cancer Cell 2016, 30, 533–547.

53. Duffy, A.G.; Ulahannan, S.V.; Makorova-Rusher, O.; Rahma, O.; Wedemeyer, H.; Pratt, D.; Davis, J.L.; Hughes, M.S.; Heller, T.; ElGindi, M.; et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. J. Hepatol. 2017, 66, 545–551.

54. Kitahara, M.; Mizukoshi, E.; Terashima, T.; Nakagawa, H.; Horii, R.; Iida, N.; Arai, K.; Yamashita, T.; Sakai, Y.; Yamashita, T.; et al. Safety and Long-Term Outcome of Intratumoral Injection of OK432-Stimulated Dendritic Cells for Hepatocellular Carcinomas After Radiofrequency Ablation. Transl. Oncol. 2020, 13, 100777.

55. Ocker, M.; Mayr, C.; Kiesslich, T.; Stintzing, S.; Neureiter, D. Immunmodulatory Treatment Strategies of Hepatocellular Carcinoma: From Checkpoint Inhibitors Now to an Integrated Approach in the Future. Cancers 2021, 13, 1558.

56. Wirth, T.; Zender, L.; Schulte, B.; Mundt, B.; Plentz, R.; Rudolph, K.L.; Manns, M.; Kubicka, S.; Kühnel, F. A telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer. Cancer Res. 2003, 63, 3181–3188.

57. Zamarin, D.; Holmgaard, R.B.; Subudhi, S.K.; Park, J.S.; Mansour, M.; Palese, P.; Merghoub, T.; Wolchok, J.D.; Allison, J.P. Localized Oncolytic Virotherapy Overcomes Systemic Tumor Resistance to Immune Checkpoint Blockade Immunotherapy. Sci. Transl. Med. 2014, 6, 226ra32.

58. Woller, N.; Gürlevik, E.; Fleischmann-Mundt, B.; Schumacher, A.; Knocke, S.; Kloos, A.M.; Saborowski, M.; Geffers, R.; Manns, M.P.; Wirth, T.C.; et al. Viral Infection of Tumors Overcomes Resistance to PD-1-immunotherapy by Broadening Neoantigenome-directed T-cell Responses. Mol. Ther. 2015, 23, 1630–1640.

59. Andtbacka, R.H.; Kaufman, H.L.; Collichio, F.; Amatruda, T.; Senzer, N.; Chesney, J.; Delman, K.A.; Spitler, L.E.; Puzanov, I.; Agarwala, S.S.; et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J. Clin. Oncol. 2015, 33, 2780–2788.

60. Heo, J.; Reid, T.; Ruo, L.; Breitbach, C.J.; Rose, S.; Bloomston, M.; Cho, M.; Lim, H.Y.; Chung, H.C.; Kim, C.W.; et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat. Med. 2013, 19, 329–336.

61. Moehler, M.; Heo, J.; Lee, H.C.; Tak, W.Y.; Chao, Y.; Paik, S.W.; Yim, H.J.; Byun, K.S.; Baron, A.; Ungerechts, G.; et al. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: A randomized multicenter Phase IIb trial (TRAVERSE). OncoImmunology 2019, 8, 1615817.

62. Puzanov, I.; Milhem, M.M.; Minor, D.; Hamid, O.; Li, A.; Chen, L.; Chastain, M.; Gorski, K.S.; Anderson, A.; Chou, J.; et al. Talimogene Laherparepvec in Combination With Ipilimumab in Previously Untreated, Unresectable Stage IIIB-IV Melanoma. J. Clin. Oncol. 2016, 34, 2619–2626.

63. Ribas, A.; Dummer, R.; Puzanov, I.; VanderWalde, A.; Andtbacka, R.H.I.; Michielin, O.; Olszanski, A.J.; Malvehy, J.; Cebon, J.; Fernandez, E.; et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. Cell 2017, 170, 1109–1119.e10.

64. Desjardins, A.; Gromeier, M.; Herndon, J.E., 2nd; Beaubier, N.; Bolognesi, D.P.; Friedman, A.H.; Friedman, H.S.; McSherry, F.; Muscat, A.M.; Nair, S.; et al. Recurrent Glioblastoma Treated with Recombinant Poliovirus. N. Engl. J. Med. 2018, 379, 150–161.

65. Dudley, M.E.; Wunderlich, J.R.; Robbins, P.F.; Yang, J.C.; Hwu, P.; Schwartzentruber, D.J.; Topalian, S.L.; Sherry, R.; Restifo, N.P.; Hubicki, A.M.; et al. Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes. Science 2002, 298, 850–854.

66. Abramson, J.S.; Palomba, M.L.; Gordon, L.I.; Lunning, M.A.; Wang, M.; Arnason, J.; Mehta, A.; Purev, E.; Maloney, D.G.; Andreadis, C.; et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): A multicentre seamless design study. Lancet 2020, 396, 839–852.

67. Locke, F.L.; Ghobadi, A.; Jacobson, C.A.; Miklos, D.B.; Lekakis, L.J.; Oluwole, O.O.; Lin, Y.; Braunschweig, I.; Hill, B.T.; Timmerman, J.M.; et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): A single-arm, multicentre, phase 1–2 trial. Lancet Oncol. 2019, 20, 31–42.

68. Alexander, M.; Culos, K.; Roddy, J.; Shaw, R.; Bachmeier, C.; Shigle, T.L.; Mahmoudjafari, Z. Chimeric Antigen Receptor T Cell Therapy: A Comprehensive Review of Clinical Efficacy, Toxicity, and Best Practices for Outpatient Administration. Transplant. Cell. Ther. 2021.

69. Jiang, Z.; Jiang, X.; Chen, S.; Lai, Y.; Wei, X.; Li, B.; Lin, S.; Wang, S.; Wu, Q.; Liang, Q.; et al. Anti-GPC3-CAR T Cells Suppress the Growth of Tumor Cells in Patient-Derived Xenografts of Hepatocellular Carcinoma. Front. Immunol. 2016, 7, 690.

70. Li, D.; Li, N.; Zhang, Y.-F.; Fu, H.; Feng, M.; Schneider, D.; Su, L.; Wu, X.; Zhou, J.; Mackay, S.; et al. Persistent Polyfunctional Chimeric Antigen Receptor T Cells That Target Glypican 3 Eliminate Orthotopic Hepatocellular Carcinomas in Mice. Gastroenterology 2020, 158, 2250–2265.e20.
71. Shirakawa, H.; Suzuki, H.; Shimomura, M.; Kojima, M.; Gotohda, N.; Takahashi, S.; Nakagohri, T.; Konishi, M.; Kobayashi, N.; Kinoshita, T.; et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer Sci. 2009, 100, 1403–1407.
72. Shi, D.; Shi, Y.; Kaseb, A.O.; Qi, X.; Zhang, Y.; Chi, J.; Lu, Q.; Gao, H.; Jiang, H.; Wang, H.; et al. Chimeric Antigen Receptor-Glypican-3 T-Cell Therapy for Advanced Hepatocellular Carcinoma: Results of Phase I Trials. Clin. Cancer Res. 2020, 26, 3979–3989.
73. Rochigneux, P.; Chanez, B.; De Rauglaudre, B.; Mitry, E.; Chabannon, C.; Gilabert, M. Adoptive Cell Therapy in Hepatocellular Carcinoma: Biological Rationale and First Results in Early Phase Clinical Trials. Cancers 2021, 13, 271.
74. Verdegaal, E.M.E.; de Miranda, N.F.C.C.; Visser, M.; Harryvan, T.; Van Buuren, M.M.; Andersen, R.S.; Hadrup, S.R.; Van Der Minne, C.E.; Schotte, R.; Spits, R.H.; et al. Neoantigen landscape dynamics during human melanoma–T cell interactions. Nature 2016, 536, 91–95.
75. Giraud, J.; Chalopin, D.; Blanc, J.-F.; Saleh, M. Hepatocellular Carcinoma Immune Landscape and the Potential of Immunotherapies. Front. Immunol. 2021, 12, 655697.
76. Butterfield, L.H.; Ribas, A.; Dissette, V.B.; Lee, Y.; Yang, J.Q.; De La Rocha, P.; Duran, S.D.; Hernandez, J.; Seja, E.; Potter, D.M.; et al. A Phase I/II Trial Testing Immunization of Hepatocellular Carcinoma Patients with Dendritic Cells Pulsed with Four α-Fetoprotein Peptides. Clin. Cancer Res. 2006, 12, 2817–2825.
77. Nakagawa, H.; Mizukoshi, E.; Kobayashi, E.; Tamai, T.; Hamana, H.; Ozawa, T.; Kishi, H.; Kitahara, M.; Yamashita, T.; Arai, K.; et al. Association Between High-Avidity T-Cell Receptors, Induced by α-Fetoprotein—Derived Peptides, and Anti-Tumor Effects in Patients With Hepatocellular Carcinoma. Gastroenterology 2017, 152, 1395–1406.e10.
78. Greten, T.F.; Forner, A.; Korangy, F.; N'Kontchou, G.; Barget, N.; Ayuso, C.; Ormandy, L.A.; Manns, M.P.; Beaugrand, M.; Bruix, J. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. BMC Cancer 2010, 10, 209.
79. Mizukoshi, E.; Nakagawa, H.; Kitahara, M.; Yamashita, T.; Arai, K.; Sunagozaka, H.; Fushimi, K.; Kobayashi, E.; Kishi, H.; Muraguchi, A.; et al. Immunological features of T cells induced by human telomerase reverse transcriptase-derived peptides in patients with hepatocellular carcinoma. Cancer Lett. 2015, 364, 98–105.
80. Sawada, Y.; Yoshikawa, T.; Nobuoka, D.; Shirakawa, H.; Kuronuma, T.; Motomura, Y.; Mizuno, S.; Ishii, H.; Nakachi, K.; Konishi, M.; et al. Phase I Trial of a Glypican-3–Derived Peptide Vaccine for Advanced Hepatocellular Carcinoma: Immunologic Evidence and Potential for Improving Overall Survival. Clin. Cancer Res. 2012, 18, 3686–3696.
81. Yoshikawa, T.; Nakatsugawa, M.; Suzuki, S.; Shirakawa, H.; Nobuoka, D.; Sakemura, N.; Motomura, Y.; Tanaka, Y.; Hayashi, S.-I.; Nakatsura, T. HLA-A2-restricted glypican-3 peptide-specific CTL clones induced by peptide vaccine show high avidity and antigen-specific killing activity against tumor cells. Cancer Sci. 2011, 102, 918–925.