


Peptide-Based Vaccines for Hepatocellular Carcinoma: A Review of Recent Advances

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Abstract: Primary liver cancer is the sixth most commonly diagnosed cancer and the third leading cause of cancer-related deaths worldwide. After surgery, up to 70% of patients experience relapses. The current first-line therapy for advanced cases of hepatocellular carcinoma (HCC) comprises sorafenib and lenvatinib administered as single-drug therapies. Regorafenib, cabozantinib, and ramucirumab are administered as second-line therapies. Recently, it has been reported that using the immune checkpoint inhibitors atezolizumab (anti-PDL1 antibody) and bevacizumab (anti-VEGF antibody) leads to longer overall survival of unresectable cases, when compared with the use of sorafenib. The role of cancer immunity against HCC has attracted the attention of clinicians. In this review, we describe our phase I/II clinical trials of peptide vaccines targeting GPC3 in HCC and discuss the potential of peptide vaccines targeting common cancer antigens that are highly expressed in HCC, such as WT-1, AFP, ROBO1, and FOXM1. Further, we introduce recent cancer vaccines targeting neoantigens, which have attracted attention in recent times, as well as present our preclinical studies, the results of which might aid to initiate a neoantigen vaccine clinical trial, which would be the first of its kind in Japan.

Keywords: common cancer antigen, cancer vaccine, glypican-3, neoantigen, personalized peptide vaccine

Introduction

In 2020, there were 907,100 new cases of primary liver cancer, and the disease was the cause of death in 8.3% of the patients.¹ These numbers make primary liver cancer the sixth most commonly diagnosed cancer and the third leading cause of cancer-related deaths worldwide. The prevalence of this disease is annually increasing owing to a higher rate of diagnosis as well as the longer life expectancy of the patient. Risk factors,² leading in most of cases to a cirrhosis state, are hepatitis B and C type (which are viruses widely spread in Asia and Africa), fatty liver disease including nonalcoholic fatty liver diseases and NASH (nonalcoholic steatohepatitis) and alcoholic liver diseases. Cases of food contamination by the mycotoxin B1 aflatoxin produced by *Aspergillus* or industrial pollution have also been reported to contribute to the development of hepatocellular carcinoma (HCC).² HCC has a high recurrence rate, ranging between 40% and 70%,³ and it is resistant against several standard therapies; therefore, it is classified as a refractory cancer. Early diagnosis leads to a 5-year survival rate higher than 70% when surgical resection or local radiofrequency ablation is applied.^{3,4} However, when the cancer is at a later advanced stage or is unresectable, the 5-year survival rate is lower than 16%. Sorafenib is the first line of treatment; it is very effective at early

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stages of the disease, but its efficacy reduces as the disease progresses.⁵ However, as sorafenib administration can lead to drug-resistance acquisition, favoring the growth of resistant tumor clone cells, the drug lenvatinib is also frequently used as the first-line treatment.⁶ Even though lenvatinib is not more efficient than sorafenib in terms of overall survival, lenvatinib is associated with a higher overall response rate and progression-free survival than sorafenib. In addition, new immunotherapy strategies are emerging as robust candidates for the treatment of HCC. Administration of small molecule inhibitors of multiple receptor tyrosine kinases in combination with immune checkpoint inhibitors (ICIs) has been reported to be effective in other cancers and is currently under investigation as a treatment option for HCC.

Immunotherapy approaches have been reported to be efficacious for various cancers.⁷ In addition, the idea of using of our own immune system to fight the disease is positively perceived and easily understood by the public. Immunotherapy is frequently used in combination with radiotherapy or chemotherapy or as the last resort if the two therapies have failed. The main immunotherapies already used in a clinical setting comprise ICIs.⁸ These compounds target immune checkpoint molecules present on the surface of cells, which physiologically restrict the risk of an auto-immune response after immune activity. However, cancer cells take advantage of these checkpoints and thus remain protected against tumor-specific T cells. PD1/PD-L1⁹ and CTLA-4¹⁰ are the most well-known checkpoint receptors and are highly expressed in the membrane of T cells and tumor cells. In cancer patients, T cells frequently remain in an “exhausted” state,¹¹ which implies that they have been activated and stimulated at a low level by the tumor antigens but could not induce a strong immune response due to the “safe” control by Treg cells. Hence, as tumor cells express immune checkpoints in high quantities, they are easy targets for ICIs. A study showed that immunotherapy can be beneficial for HCC, as administering a combination of atezolizumab (anti-PDL-1) and bevacizumab (anti-VEGF) to unresectable HCC cases led to increased overall survival, longer median survival, and increased overall response rate in comparison with administering sorafenib.¹²

To further understand immunotherapy and its efficacy, it is necessary to consider the inter-individual differences in disease responses. Each person has their own “immunity ID” composed of the Human Leukocyte Antigen (HLA) system,¹³ a complex expressed at the surface of

most molecules of MHC I, encoded by 6 different alleles, and MHC II, encoded by eight different alleles. The MHC system is the main guard against self-aggression of our bodies. Peptides that result from degradation of self or exogenous proteins are presented by MHC and recognized by some T cells, using a surface receptor system named T-cell receptor (TCR). In the case of a matched MHC-peptide-TCR combination, T cells are activated and start an expansion phase, which takes several days. CD4 T cells recognize MHC class II and help other immune cells by enhancing their immune response secretion factors, such as IFN- γ and interleukins.¹⁴ CD8 T cells present cytotoxic activity,¹⁵ which destroys the cells presenting the recognized peptides. Previous studies have reported the presence of antigens specifically expressed by cancer cells, termed “cancer antigens,” which have the potential to be the targets that are recognized by T cells, and used for cancer treatment. In this review, first, we summarize the “cancer antigens” expressed in HCC and then review the future development and potential of cancer vaccines targeting these cancer antigens based on our experience in clinical and preclinical studies.

Cancer Antigen Classification

The most commonly used cancer antigen classifications are listed in Table 1.^{16–25} To date, more than 200 cancer antigens have been identified. The antigens and their T cell epitope presented were obtained from TANTIGEN 2.0: Tumor T-cell Antigen Database (met-hilab.org) and Cancer antigen peptide database (<https://caped.icp.ucl.ac.be/>).

Common cancer antigens (CCAs) can usually be organized into three categories: tumor-associated antigens (TAAs), antigens derived from gene mutations in tumors, and virus-derived antigens.²⁶ TAAs are usually overexpressed in tumors compared with those in normal tissues; therefore, they are also used as diagnostic markers. This is the case for prostate-specific antigen, carcinoembryonic antigen, and alpha-fetoprotein levels in serum. Since some TAAs are also expressed in normal cells at a lower level, targeting them with immunotherapy presents the risk of triggering an auto-immune response. Tumor-specific antigens (TSAs) originate from oncogenic drivers with non-synonymous mutations, leading to modified peptides or “neoantigens.” Therefore, TSAs are expressed exclusively in tumors and are not found in healthy tissues. TSAs serve as ideal targets for diagnosis and immunotherapy because of their tumor specificity and the absence of immune tolerance. The interest of using the mutated

Table I The Classification of Cancer Associated Antigens and Their Expression in Cancers

Category	Antigens	Antigens Characters	HCC Expression	Ref	
Cancer/Testis (CT) antigens	MAGE	These antigens are expressed in cancer tissues and normal tissues only in testis, ovary, and placenta. Although the frequency of patients who have the expression of these antigens is not high, they also are expressed in a variety of cancers, therefore, may be applied to many cancers as a target of anti-tumor immunity.	X	[16]	
	NY-ESO-1		X	[17]	
Cancer/Fetal antigens	CEA	These antigens are expressed in cancer tissues and only normal fetal tissues. CEA was first discovered as cancer fetal antigen, and used as serum marker for cancer diagnosis. Not only cancer patients, these serum biomarkers are detected in other diseases, such as inflammation and diabetes.	X	[18]	
	GPC3		X	[35]	
Differentiation antigens	Tyrosinase	Differentiation antigens are specifically expressed on cancer cells and in normal tissues where cancer develops. MART-I was well known differentiation antigen that was first identified in melanoma by Boon et al. If these antigens are also expressed in normal organs, it may cause damage to those organs.			
	gp100				
	Melan-A (MART-I)				
	PSA		X	[19]	
Antigens associated with genetic abnormalities		Cancer is a disease caused by the accumulation of genetic abnormalities, and the gene products derived from these abnormalities have been identified as cancer antigens. Genetic abnormalities that occur infrequently are not useful in terms of versatility, but in recent years, these abnormalities have attracted considerable attention as cancer-specific mutant antigens (neoantigens).			
	Somatic mutation	TP53	Antigens associated with somatic gene mutations in cancer	X	[20]
		EGFR		X	[21]
	Over expression (copy-number amplification)	HER2	Antigens due to the amplification or overexpression of gene products	X	[22]
		hTERT		X	[23]
	Alternative splicing	Survivin-2B	Antigens associated with alternative splicing	X	[24]
Fusion genes	bcr/abl	Antigens associated with fusion genes			
Viral antigens	HPV E6	Antigens derived from viral genomes. Human T-cell leukemia virus (HTLV), Epstein-Barr (EB) virus, which is associated with Burkitt's lymphoma and head and neck cancer, and human papillomavirus (HPV) are known to be associated with carcinogenesis, and to be recognized by the immune system as cancer rejection antigens.	X	[25]	
	HPV E7		X	[25]	

antigen to increase the immune response in comparison with the wild-type antigen for diagnosis or immunotherapy has been known for a long time.²⁷ However, neoantigens

are, in general, patient-specific as a result of the tumor heterogeneity and the inter-individual mutations observed in all cancers. Developing and using therapy approaches

targeting neoantigens have several important requisites: 1) Neo-peptide sequences need to be predicted using sequencing technology and bioinformatics approaches; 2) The binding affinity for MHC molecules needs to be determined (increased or decreased); 3) The power of the immune response or antigenicity needs to be decreased. The disadvantages of this strategies are the high tumor mutation burden and the low number of neoantigens.

Another type of TSAs are oncogenic viral antigens. Various viruses successfully infect human cells in a persistent manner and sometimes may integrate in the host DNA, such as the human papillomavirus or the Epstein–Barr virus, or remain in the cytoplasm as RNA forms, such as the hepatitis C virus. These viruses frequently inactivate the guardians of the genome p53²⁸ and Rb,²⁹ preventing apoptosis and causing the immortalization of cells. This could lead to the production of virus-induced tumor cells. These infected cells produce specific proteins and peptides that are recognized as foreign antigens, such as HBsAg (Hepatitis B),³⁰ p24 (HIV),³¹ and pp65 (cytomegalovirus).³²

Common Cancer Antigens in HCC

In the following section, we describe some well-studied CCAs, such as GPC3, AFP, WT-I, FOXM1, and ROBO1, which are overexpressed in HCC, which could be used for peptide vaccines using predicted or isolated peptides. CAAs in HCC are overexpressed, meaning that in comparison with healthy tissues, the tumor cells produce a lot of mRNA and proteins of these CAA. Consequently, it is considered that around 1 peptide for 10,000 degraded proteins is presented to MHC³³ and that the production of MHC-I peptides is correlated to the mRNA quantity, it is likely that overexpressed CAAs is good to be considered as onco-antigen candidates for cancer vaccine.³⁴

Glypican-3 (GPC3)

In physiological conditions, GPC3 regulates cell division and growth regulation through binding to growth factors via heparan sulfate chains.^{35,36} In addition, GPC3 is involved in pathological conditions, such as the Simpson-Golabi-Behmel syndrome, a genetic disease characterized by growth anomalies. Furthermore, GPC3 is highly expressed in some cancers, such as melanomas,³⁷ ovarian clear cell carcinoma,³⁸ lung squamous cell carcinoma,³⁹ hepatoblastoma,⁴⁰ nephroblastoma,⁴¹ yolk sac tumor,⁴² and hepatocellular carcinoma (HCC).³⁵ Importantly, high GPC3 expression levels are associated with a poor

prognosis in HCC.⁴³ Because the expression of GPC3 is not observed in most normal tissues, except for the placenta, fetal liver, and fetal kidney, GPC3 is considered an ideal tumor-associated antigen to develop a cancer vaccine.⁴⁴

Alpha Fetoprotein (AFP)

AFP is highly expressed in fetal tissues, but it is barely detected in adult tissues.⁴⁵ AFP expression is commonly used for testicular cancer diagnosis and HCC, with levels higher than 400 µg/mL for an HCC diagnostic.⁴⁶ As AFP is specifically overexpressed in HCC,⁴⁷ targeting this antigen stands as a promising strategy. Recently, the interest of using high-avidity TCRs to induce an efficient anti-tumor response in HCC has been recently studied in a Japanese Phase I clinical trial.⁴⁸ In this study, two AFP-derived peptides (AFP357 and AFP403) were injected into 15 patients with HCC, the toxicity of the treatment and the tumor evolution were monitored, and the high-avidity TCR of a patient who responded positively was cloned. Later on, the same team studied the potential of 14 predicted and AFP-derived peptides restricted to HLA class II, using the peripheral blood mononuclear cells from 47 patients with several types of liver cancer, including HCC.⁴⁹ Four peptides showed interesting immune responses. Among them, AFP1 was the most active and presented a strong avidity for HLA-DR. Furthermore, patients expressing this peptide presented an increased survival. After HCC treatment, an increased frequency of peptide-specific T cells was observed in some patients with HLA-DRB1*1502, *0405, and *0901 alleles, demonstrating the interest of not only focus on class I but also research on the class II for a successful immunotherapy. Finally, it is important to mention that the AFP1 peptide is also presented by MHC class I, thus indicating the importance of developing immunotherapy considering the whole immune system.

Wilms' Tumor-I (WT-I)

Wilms' tumor 1 (WT1) antigen, a part of a transcription factor encoded by a 50 kb gene that was discovered in 1990, is important for the development of the urogenital system.⁵⁰ As a result of different mRNA splicing events, alternative start codons, and RNA editing, there are 24 isoforms of WT1, which have different functions. Similar to GPC3, WT1 is expressed in fetal tissues but not in healthy adult tissues. However, its expression is observed

in chronic hepatitis and cirrhotic liver, both of which are associated with HCC. WT1 overexpression is oncogenic⁵¹ and is associated with poor prognosis in breast cancer⁵² and leukemia.⁵³ In addition, a recent report detected the overexpression of the WT-1 factor in 95% of the tumors with worst prognostic analyzed.⁵⁴ Furthermore, WT-1 overexpression is known to promote cell dedifferentiation and resistance to chemotherapy.⁵⁵

Forkhead Box M1 (FOXMI)

FOXMI is a proliferation-associated transcription factor that has four different isoforms. FOXMI expression is a sign of active proliferation, such as that occurring in progenitor cells or regenerating tissues and also in several cancers.⁵⁶ Overexpression of FOXMI is associated with gene instability, cessation of senescence, and resistance to endocrine therapies in breast cancers.⁵⁷ In addition, FOXMI upregulates CCNB1, enhancing the proliferation of cells.⁵⁸ FOXMI is overexpressed in HCC when compared to that in non-tumor tissue, and its expression levels are correlated with tumor stage and tumor size.⁵⁹ Hence, targeting FOXMI is a suitable approach for a peptide vaccine. Some experiments have already been performed using pulsed dendritic cells with a cytoplasmic transduction peptide fusion protein of FOXMI to evaluate the induced anti-tumor activity in HCC. In comparison with the control, a tumor regression with specific CTL activity was observed in the peptide condition.⁶⁰ Furthermore, another study demonstrated that the use of FOXMI predicted peptides to stimulate specific CTLs in vitro with human cells, confirming the potential for targeting this antigen.⁶¹

Roundabout Homolog-1 (ROBO-1)

ROBO-1 was initially discovered in *Drosophila*,⁶² after which Robo homologs have been discovered in several species, including humans. Robo belongs to the immunoglobulin superfamily of cell adhesion molecules, and its ligand is SLIT. Physiologically, these receptors are important for axon guidance in the ventral midline of the neural tube during neural development. ROBO-1 has been described to have an antitumor effect in breast cancer and pancreatic ductal adenocarcinoma,⁶³ but it is overexpressed in HCC. The overexpression of ROBO-1 on the cell surface in HCC makes it an interesting target for CAR-T cell therapy as well as peptide-based vaccines.⁶⁴

Development of Cancer Vaccine for HCC

Historically, large-scale vaccination started after Edward Jenner's experiment in the 18th century. Owing to vaccination, poliomyelitis, rinderpest and smallpox has been almost eradicated, and the number of people infected from diphtheria, tetanus, or pertussis has greatly diminished. The discovery of pathogens and a deep understanding of the functioning of the immunological system have helped unravel the mechanisms underlying protection immunity and the development of vaccines. How to efficiently establish a long-term and potent immunological memory against pathogens has been a critical research topic for a long time, including matters such as what kind of vectors and adjuvants, as well as the optimal type of vaccines, can best induce an effective immune response.⁶⁵ DNA vaccines, consisting of a DNA plasmid encoding a certain antigen, have gained increasing attention over time. DNA vaccines can be directly injected into a specific site of the body, where APCs or other targeted cells take up the plasmid DNA and produce and expand the antigen. Another type of DNA vaccine that has been used for vaccination is the adenovirus, which allows the antigen genes to integrate into the host genome, and hence, the replication and expression of the gene at the same time as the cells do. However, these strategies involving the integration of a gene into the host genome convey the risk of inducing a mutation in the host genome. Hence, vaccines based on the transient expression of genes, inactivated pathogens, RNA, antigen proteins, or antigen peptides have been recently developed. Materials that are used as adjuvant for vaccine were summarized in Table 2, which is adapted from the review of Bonam et al,⁶⁵ with new additions post-2015 made with the selection list using google patents and the terms, "adjuvant", "vaccine" and "cancer", granted between 2015 to 2021 in US (USA) and EP (European).

In the 20th century, the identification of "cancer antigens" led to the development of vaccines to prevent, hopefully cure, cancer. Cancer vaccines need to induce a potent CTL response to regress tumor cells. In general, the antigenicity of tumor cells and their antigens is weaker than that of pathogens as a result of them originating from autologous cells. The development of cancer vaccines presents additional obstacles, including the chronic disease status of cancer patients, heterogeneity of tumor cells, and immunological tolerance against autologous tissues. A great variety of approaches have been developed to overcome these issues.⁶⁶ Peptide vaccines are one of the

Table 2 Adjuvants Used for Vaccination

Categories		Year filed/ published)	Composition	Patent number	Assignee
Bacteria	toxin	1993	Vaccine comprises different toxin antigens	US 5182109A	National Institute of Health;The Kitasato Institute, both of Tokyo (JP)
		2021	Tetanus toxoid and CCL3 to improve DC vaccines	US-11071777-B2	Duke University
	bacteria body	2005	TB vaccine that comprises mycobacterial	PCT/EP00/01046	Eurocine AB, Stockholm, Solna (SE)
		2010	Vaccine comprises different <i>Streptococcus pneumoniae</i> capsular saccharide conjugates	PCT/EP08/57998	GlaxoSmithKline Corporate Intellectual Property, North Carolina (USA)
		2021	Lactic-acid-bacteria-containing composition for treating hpv infection and/or hpv-associated tumors, and mucosal immunity-inducing agent	EP-3254693-B1	The University of Tokyo, Japan As Represented By Director General Of National Institute Of Health Sciences
		2020	Immunomodulatory composition comprising bifidobacteria	US-10668117-B2	Dupont Nutrition Biosciences Aps
Cytokine		2013	Supernatant collected from stimulated cultured human lymphocytes	PCT/US2004/005152	HasumiLlc (Dba Shukokai International) New York (USA)
		2003	Nerve growth factor acts as an adjuvant to enhance effectiveness of vaccine	PCT/US98/08652	Protection Unlimited, Inc., Wilmington, DE (USA)
DNA	CpG (TLR9 agonist)	2002	Unmethylated CpG ODN and non-nucleic acid adjuvant	US 6,406,705 B1	University of Iowa Research Foundation, Iowa City (USA); Coley Pharmaceutical GmbH, Langenfeld (DE); Ottawa Health Research Institute, Ottawa (CA)
		2004	Vaccine comprises fusion peptide synthetically derived from cytomegalovirus with CpG ODN as an adjuvant	US 2004/0101534 A1	Rothwell' Figg' Ernst & Manbeck, P.C. Washington (USA)
		2005	Immune adjuvant comprising approximately 10-bp single-stranded oligonucleotide with a CpG motif; produced by treatment of bacterial DNA with endonuclease	US 6,881,561 B1	Cheil Jedang Corporation, Seoul (KR)
		2010	CpG DNA	US 7,749,979 B2	National Pingtung University of Science and Technology, Pingtung County (TW)
	Interferon-stimulating gene 15	2020	ISG15 gene nucleic acid molecule	US-10792358-B2	The Trustees Of The University Of Pennsylvania
RNA	polyI:C.LC (TLR3 agonist)	2011	The effect of Poly-I:CLC is a more direct antiviral and anti-neoplastic effect mediated by interferon-inducible nuclear enzyme systems.	(US) EPI778186	Oncovire Inc.(USA)

(Continued)

Table 2 (Continued).

Emulsion	oil	1996	W/O emulsion with different surfactant and polymer ratios	EP 0781 559 A2	Juridical Foundation, The Chemo- Sero- Therapeutic Research Institute Kumamoto-ken (JP)
		2005	Vaccine comprises W/O/W-type oil adjuvant	US 2005/0158330 A1	NOF Corporation, Tokyo (JP); Juridical Foundation, Kurnarnoto-shi (JP)
		2012	O/W emulsion comprises metabolizable oil, tocol, emulsifying agent with various antigens and methods of preparation	PCT/EP2006/069979	GlaxoSmithKline Biologicals S.A., Rixensart (BE)
	Aluminium	2013	Provides improved methods for producing the aluminium adjuvant AlPO ₄	US 8,540,955 B2	Wyeth LLC, Madison (USA)
		2013	Preparation of freeze-dried vaccine comprising an aluminium salt adjuvant, a recombinant <i>Clostridium botulinum</i> neurotoxin protein, and a glass-forming agent	PCT/US2008/057355	The Regents of the University of Colorado, A Body Corporate, Denver (USA)
		2021	Effective amount of an autoimmune antigen and an anti-inflammatory cytokine with an aluminum-based carrier	US-10940200-B2	East Carolina University
	saponin	1998	Comprises saponin component from the bark of <i>Quillaja saponaria</i> Molina as an immune adjuvant	PCT/KR96/00053	LG Chemical Ltd., Seoul, (KR)
surfactant	2015	Oil-based adjuvants comprise a plant-derived surfactant, such as gum arabic, an aqueous component, and an oil	PCT/US2013/034372	Kansas State University Research Foundation, University of Tennessee Research Foundation, Manhattan (USA)	
Glycolipid	a-GalCer	2010	a-GalCer-containing triazole moiety at the amide position	PCT/KR2007/006889	SNU R&DB Foundation, Seoul (KR)
	a-GalCer	2010	Synthetic a-C-GalCer	US 7,771,726 B2	New York University, New York (USA); The Research Foundation of the City University of New York, New York(USA); Aaron Diamond Aids Research Center, New York (USA)
Peptidoglycan	TLR2 agonist	2014	FMDV capsid proteins VP1 and VP3 activate TLR2	US 8,795,678 B2	Academia Sinica, Taipei (TW)
	TLR2 agonist	2016	Lipidated cysteine-based triazoles as vaccine adjuvants covered both therapeutic and prophylactic vaccines against bacterial, viral, and protozoan infections, and cancer	US2016/0200758A1	Council of Scientific and Industrial Research, Delhi (India)
	Glycan	2021	Glycan-dependent immunotherapeutic molecules	US-10925972-B2	The Regents Of The University Of California
Viral (Viral protein)	viral membrane	2009	Membrane protein from pathogens or tumor cells with amphiphilic adjuvant	PCT/NL2004/000437	Bestwil Holding B.V., Amsterdam (NL)
	influenza	2009	Vaccine comprises influenza viral antigen, QS21, and sterol	PCT/EP05/05786	SmithKlineBeecham Corporation Corporate Intellectual Property, Pennsylvania (USA)
	dengue viral	2011	Inactivated dengue virus antigen in combination with aluminum-free adjuvant	PCT/EPI0/51882	GlaxoSmithKline Biologicals S.A. Rixensart (BE)
	dengue viral	2020	recombinant subunit NS3 proteins + a purified whole inactivated dengue virus immunogenic composition	US-10716843-B2	The United States Of America As Represented By The Secretary Of The Navy, The United States Of America As Represented By The Secretary Of The Army
	HBV	2014	HBV antigen that includes cysteine	US 8,624,004 B2	GlaxoSmithKline Biologicals S.A., Rixensart (BE)

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Table 2 (Continued).

Other	latex polymer	2010	Composition comprises an adjuvant such as inverse latex or polymer mixed into an antigenic medium	PCT/FR08/51807	Air Liquide USA LLC, Intellectual Property, Houston, Texas (USA)
	long-chain hydrocarbons	2014	Short and long-chain hydrocarbons with <i>Coccidia</i>	PCT/FR2011/050069	Société d'Exploitation de Produits pour les Industries Chimiques, Paris (FR)
	liposome	2014	Liposomal vaccine formulations comprising an adjuvant and an immunogen for immunotherapy	US 8,889,616 B2	Oncothyreon Inc., Seattle (USA)
	Non-conventional liposomes	2021	saponin-free liposome	EP-3325015-B1	Zoetis Services LLC
	PRRagonist	2021	Use of Diprovocims, a TLR agonists	US-11040959-B2	The Board Of Regents Of The University Of Texas System, The Scripps Research Institute
	CD40 ligand	2021	Vaccines with CD40 ligand as an adjuvant	US-10925961-B2	The Trustees Of The University Of Pennsylvania, Inovio Pharmaceuticals, Inc.
	Nanoparticles	2019	Synthetic nanoparticles for delivery of immunomodulatory compounds	US-10300145-B2	Massachusetts Institute Of Technology
	TLR7/8 agonist	2020	Alkyl chain modified imidazoquinoline TLR7/8 agonist compounds and uses thereof	US-10618896-B2	Dynavax Technologies Corporation
		2013	Process for stabilizing a vaccine composition with a stabilizer	EP 2 589 392 A2	Sanfi Pasteur, Lyon (FR)

Note: Adapted from *Trends Pharmacol Sci.* 38(9). Bonam SR, Partidos CD, Halmuthur SKM, et al. An overview of novel adjuvants designed for improving vaccine efficacy. *Trends Pharmacol Sci.* 771–793, Copyright (2017), with permission from Elsevier.⁶⁵

most popular cancer vaccines. They consist of a peptide or a mix of linked or free peptides, combined with adjuvants that stabilize them and increase their efficacy. The specific peptide most suitable for developing a cancer vaccine is dependent on the specific type of cancer and the immunological characteristics of the patient. Despite this, peptide vaccines are frequently used for disease treatment because of their simplicity. Recent developments focused on various ways to target liver cancer. We can mention the use of a peptide vaccine cocktail⁶⁷ with several antigens to increase the efficacy of antitumor response. The use of dendritic cells, an important platform for T cell activation, as vaccines has also demonstrated interesting results in several studies [Table 3](#).^{68–74} Some other teams focused on the use of oncolytic viruses⁷⁵ with results to be demonstrated in clinical trials in the future. Cold-inducible RNA binding protein⁷³ or Tumor RNA-Loaded Lipid Nanoparticles⁷⁴ have also recently been study and demonstrated potential, especially in use with ICI. At the time this review was written, a database search (<https://clinicaltrials.gov/>) using the key words “cancer” and “peptide vaccine” retrieved 282 completed clinical trials, 35 actives (not recruiting), and 73 currently recruiting clinical trials. A narrower search using the keyword “hepatocellular

carcinoma” identified six studies, which are summarized in [Table 4](#).

Here, we summarize the results of our clinical trial targeting GPC3 and introduce our recent studies on the development of cancer vaccines targeting CCAs and neoantigens.

Phase I and II Clinical Trial of Peptide Vaccine Targeting Glypican-3

Several clinical trials have been performed and were simply summarized in the [Figure 1](#). In 2011, we published the results of a peptide vaccine against glypican-3, which is frequently upregulated in HCC.⁷⁶ From February 2007 to November 2009, 33 patients from the National Cancer Center Hospital East (Kashiwa, Japan) with advanced HCC were enrolled in a phase I trial to study the safety and immunogenic response of the peptide vaccine.⁷⁷ Half of the patients with the allele HLA-A24 were vaccinated three times, every two weeks using an escalation method with the GPC3_{298–306} peptide (EYILSLEEL), and patients with the allele HLA-A02 vaccinated with the GPC3_{144–152} peptide (FVGEFFTDV). Both HLA types were chosen due to their high frequency in the population, with approximately 60% of

Table 3 Examples of Other Anti-Tumor Vaccine Methods

Therapy Used	Ref		Observations
Resection + DC vaccine/CIK	[68]	Human	Increased mean survival of patients with advanced (stage III) HCC
			Demonstration of the safe use of DCs
Resection + DC vaccine/CIK	[69]	Human	Prevented recurrence 1 y after operation
			Improved RFS and OS
Ex-vivo prepared DCs (lysate pulse, cytokine cocktail stimulation)	[70]	Human	1- and 2-year survival rates were significantly prolonged
			Disease control of 67%
Autologous DC pulsed with liver tumor cell line lysate	[71]	Human	Safe and well tolerated method
			Immune response observed with a disease control rate of 28%
Intratumoral injection of autologous DCs	[72]	Human	Up to 17 days of the persistence of DCs in tumor
			Induction of tumor specific lymphocytes
			No clinical benefits observed
TLR4 ligand cold-inducible RNA binding protein + ICI	[73]	Mice	Enhanced therapeutic efficacy of ICI
			Strong exhaustion phenotype limitation of the intratumoral lymphocytes
Tumor RNA-Loaded Lipid Nanoparticles	[74]	In vitro/ Mice	DC maturation, CD8+ and CD4+ activation
			Preventive and therapeutic effects in mice

the Japanese population bearing HLA-A24 and 40% of Caucasian population bearing HLA-A02. This study showed, for the first time, a correlation between the frequency of peptide-specific cytotoxic T lymphocytes and the overall survival of the patients as well as the safety of the use of the GPC3 peptide vaccine (Figure 2A).

Since radiofrequency ablation leads to an enhancement of specific T cells against HCC-associated antigens or GPC3, the same team decided to perform a single-arm Phase II study, in which 41 patients were enrolled to receive a GPC3-derived peptide vaccine as adjuvant therapy.⁷⁸ Although the primary endpoint was not successfully reached for 1- and 2-year recurrence rates, the presence of GPC3 peptide-specific CTL responses was detected in 35 of the 41 patients. It is interesting to note that two patients who presented GPC3-positive primary tumors and experienced relapses did not present GPC3 in the recurrent tumors. Indeed, the GPC3 peptide-specific CTL successfully killed the tumor cells expressing GPC3, leaving the GPC3-negative cells to expand by selective pressure. This observation highlights the necessity of using different antigen peptides simultaneously to increase the

chance of tumor regression and reduce the risk of recurrence. Another important observation was the exhausted state of the specific CTLs post-vaccination, determined by PD-1 or CTLA-4 expression, which can be overcome by ICIs. Finally, another pilot study about the use of the GPC3 peptide vaccine against advanced HCC has been performed and it demonstrated the intra-tumoral infiltration of GPC3-specific peptides CTLs, the correlation between the CTLs frequency and the overall survival, the similar TCRs repertoire by comparing tumor infiltrated cells and PBMCs and they managed the isolation of GPC3 peptide-specific CTL clones.⁷⁹

Based on the positive results on this vaccine in the previous phase I clinical trial, we attempted to investigate the underlying immune mechanisms. For this purpose, five patients with HLA-A02 and six patients with HLA-A24 who were vaccinated with the same peptides previously described were selected. Most of the selected patients showed progressive disease and a poor response to sorafenib. Before- and after-vaccination tumor biopsies revealed the presence of GPC3-specific infiltrated CTLs, which was correlated with a better overall

Table 4 Clinical Trial of Cancer Vaccines Targeting HCC

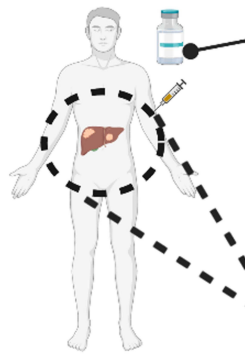
Target	Phase	Start - End		Number of Patients	Peptide	Methods/Combination	Descriptions
Ras mutation	2	Oct-97	May-07	17	Tumor Specific Mutated Ras Peptides	IL2 or GM-CSF	Phase II trial to study the effectiveness of vaccine therapy plus interleukin-2 and/or sargramostim in treating adults who have metastatic solid tumors.
AFP	I and 2	Jan-01	Oct-08	33	AFP - Four immunodominant HLA-A*0201-restricted peptides [hAFP137-145 (PLFQVPEPV), hAFP158-166 (FMNKFIYEI), hAFP325-334 (GLSPNLNRFL), and hAFP542-550 (GVALQTMKQ)]	Dendritic cells	Phase I/II trial to study the effectiveness of vaccine therapy in treating patients who have liver cancer.
16 common cancer antigens	I and 2	Sep-17	Dec-19	22	16 newly discovered and overexpressed tumor-associated peptides	Novel RNA	New cancer vaccine called IMA970A combined with CV8102, a new adjuvant for the treatment of liver cancer (hepatocellular carcinoma).
AFP	I and 2	Jul-99	Jun-02	6	AFP - Four immunodominant HLA-A*0201-restricted peptides [hAFP137-145 (PLFQVPEPV), hAFP158-166 (FMNKFIYEI), hAFP325-334 (GLSPNLNRFL), and hAFP542-550 (GVALQTMKQ)]	Intradermal	Phase I/II trial to study the effectiveness of vaccine therapy in treating patients who have liver cancer.
DNAJBI-PRKACA	I	Apr-20	-	Recruiting for 12	DNAJBI-PRKACA	Nivolumab and Ipilimumab	Primary objective of the trial is the safety and tolerability of administering a vaccine.
VEGRF1, VEGFR2	I	2007	2013	Recruiting for 9	VEGRF1, VEGFR2		The purpose of this study is to assess toxicities of angiogenic peptide vaccine therapy in treating HLA-A*2402 restricted patients with advanced hepatocellular carcinoma.

survival. The autopsy of a patient who received two injections showed a strong immune response with infiltrating CD8+ CTLs and tumor lysis. Although the disease continued to progress, the trial proved that an immune response was induced. A clone was successfully isolated for further analysis and experiments, including development of clinical TCR-engineered T cells (Figure 2B). Moreover, we have reported important observations in a phase I clinical trial of a GPC3 peptide vaccine against advanced pediatric solid tumors, including malignant hepatoblastoma. In this clinical trial, seven of 18 patients presented hepatoblastoma with uniform expression of GPC3. Of this cohort, two

patients were not evaluated, and the rest showed complete responses (CR) and did not recur for more than six years (Figure 2C). These clinical trials stand as a proof of concept that peptide vaccines targeting GPC3 are an adequate therapy to prevent relapse and have contributed to a further understanding of the mechanisms of action of the vaccine.⁸⁰ The number of patients who entered and received GPC3 peptide vaccine was however insufficient for the consideration of the efficacy of peptide vaccine against HCC. Finally, a proof-of-concept was successfully performed using glypican-3 and additional peptides, thus paving the way for treatment using various peptides and antigens.

Clinical Trial of GPC3 peptide vaccine

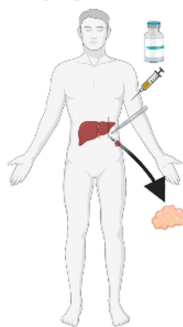
1. Phase I Clinical trial of GPC3 peptide vaccine



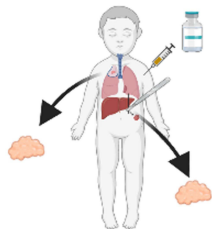
Vaccination with montanide and peptide for HLA-A24 patient: EYILSLEEL for HLA-A2 patient: FVGEFFTDV



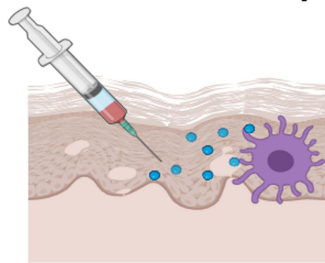
2. Phase II Clinical trial of GPC3 peptide vaccine



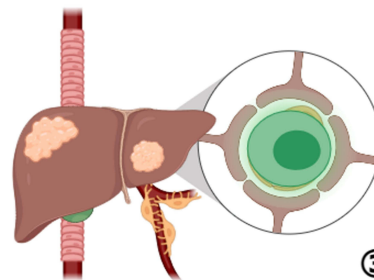
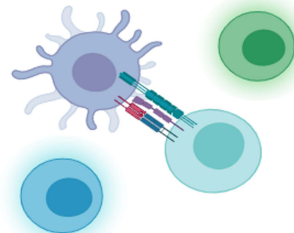
3. Phase I Clinical trial of GPC3 peptide vaccine



① Intra dermal administration of GPC3 derived peptides



② Activation GPC3-specific CD8⁺ T cells



③ Circulation and infiltration into tumor tissues

Figure 1 Scheme of our peptide vaccine trial targeting GPC3. Pictures show a series of steps to synthesize the vaccine. A24- or A2-restricted short peptides (bottle with pink label) were well mixed with montanide (bottle with white label) to prepare emulsions, and then administered to the patient intradermally. To induce a peptide-specific immune response, a series of physiological immune systems may occur in vivo (lower schemes). ① Antigen presenting cells (APCs), such as dendritic cells or Langerhans cells located in the intradermis, take up peptides of GPC3, then migrate to draining lymph nodes, where they mature. ② In draining lymph nodes, APCs present the peptides to GPC3 reactive CD8⁺ T cells and induce their activation and expansion. Activated CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTLs), infiltrate into the systemic circulation, and infiltrate into tumor tissues, resulting in tumor rejection. Using these peptide vaccines targeting GPC3, we performed phase I/II clinical trials (left figure): 1. Phase I clinical trial of GPC3 peptide vaccine in advanced HCC patients. 2. Phase II study of GPC3 peptide vaccine as an adjuvant therapy for HCC patients who underwent surgery or radiofrequency ablation. 3. Non-randomized, open-label, phase I clinical trial of GPC3 peptide vaccine in pediatric solid tumors.

Personalized Vaccine Targeting Common Cancer Antigens

The peptide vaccine using GPC3 was insufficient to induce a complete regression of the tumors. We are currently developing novel cocktail vaccines targeting various

TAAAs expressed in HCC, such as FOXM1, AFP, ROBO1, WT-1, and GPC3. As mentioned above, TAAAs are frequently overexpressed in tumor tissues, but with their efficacy is debatable; as they have an autologous origin, they are not completely considered abnormal by

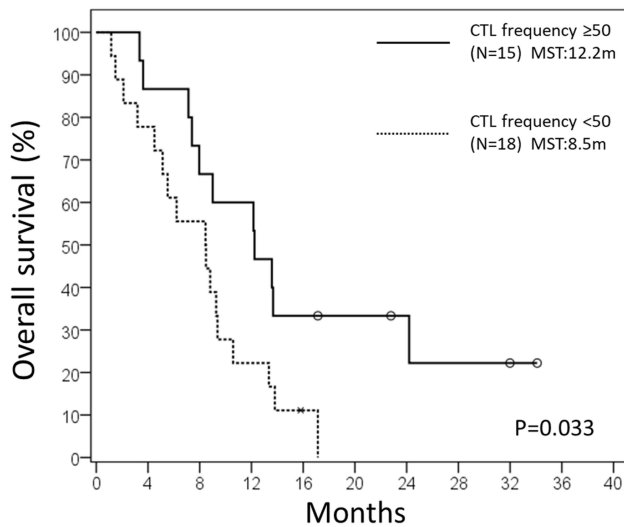
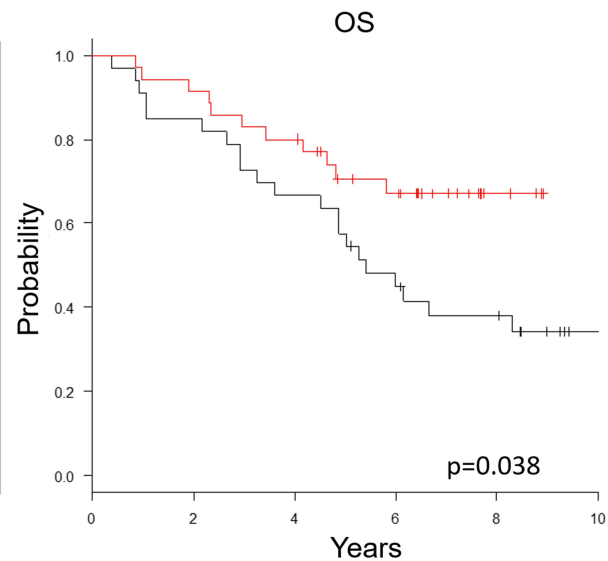
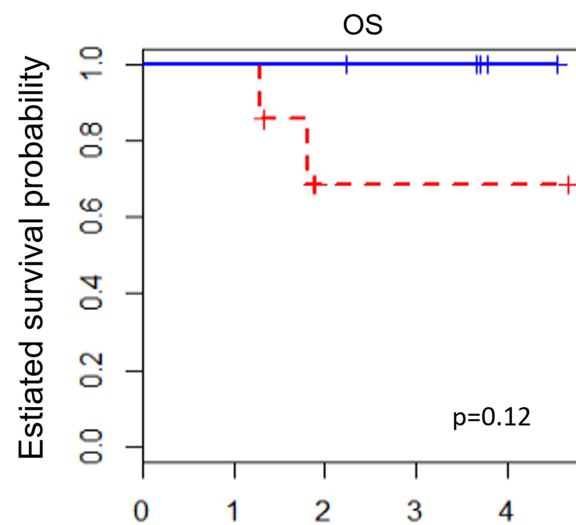
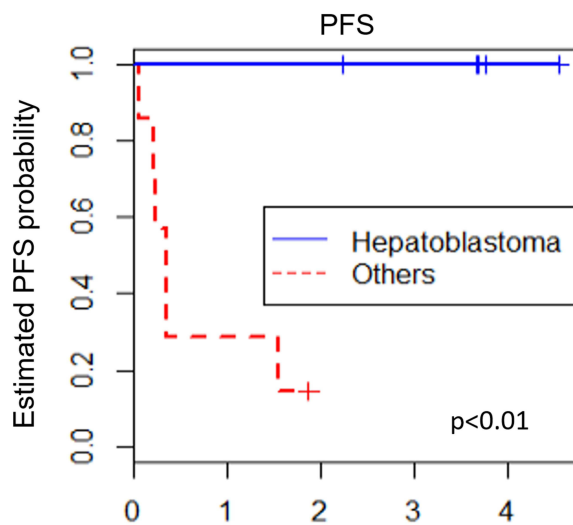
A Phase I clinical trial of GPC3 peptide vaccine**B** Phase II clinical trial of GPC3 peptide vaccine**C** Phase I clinical trial of GPC3 peptide vaccine against refractory pediatric solid tumor

Figure 2 Overall survival (OS) or Progress free survival (PFS) of patients who had been experienced with vaccine in phase I/II clinical trials of GPC3 peptide vaccine. The details are shown in Figure 1. **(A)** Kaplan–Meier curves for OS of advanced HCC patients in a phase I clinical trial of GPC3 peptide vaccine.⁷⁷ Patients with GPC3-specific CTL frequencies ≥ 50 (bold line) had a longer survival than those with GPC3-specific CTL frequencies < 50 (dotted line) ($P = 0.033$). **(B)** Kaplan–Meier curves for OS in a phase II clinical trial of adjuvant GPC3 peptide vaccine.⁸⁰ Among patients with GPC3-positive HCC, patients that underwent surgery and were vaccinated (red line) tended to have longer recurrence-free and overall survival rates than those who underwent surgery alone (black line). **(C)** Kaplan–Meier curves for PFS and OS in a phase I clinical trial of GPC3 peptide vaccine with pediatric solid tumors.⁹³ Hepatoblastoma patients in the partial remission group (blue line) showed longer PFS and OS than those with other pediatric solid tumors (red line).

Abbreviation: MST, median survival time.

the immune system. In addition, the risk of autoimmunity is increased. Hence, TAA-based peptide vaccines should be carefully developed, and after having performed clinical trials with GPC3, we wanted to explore other CAAs

expressed in HCC to increase the efficacy spectra to kill tumor cells, therefore we have now started to research about. The scheme for the development of vaccines targeting common cancer antigens is shown in Figure 3.

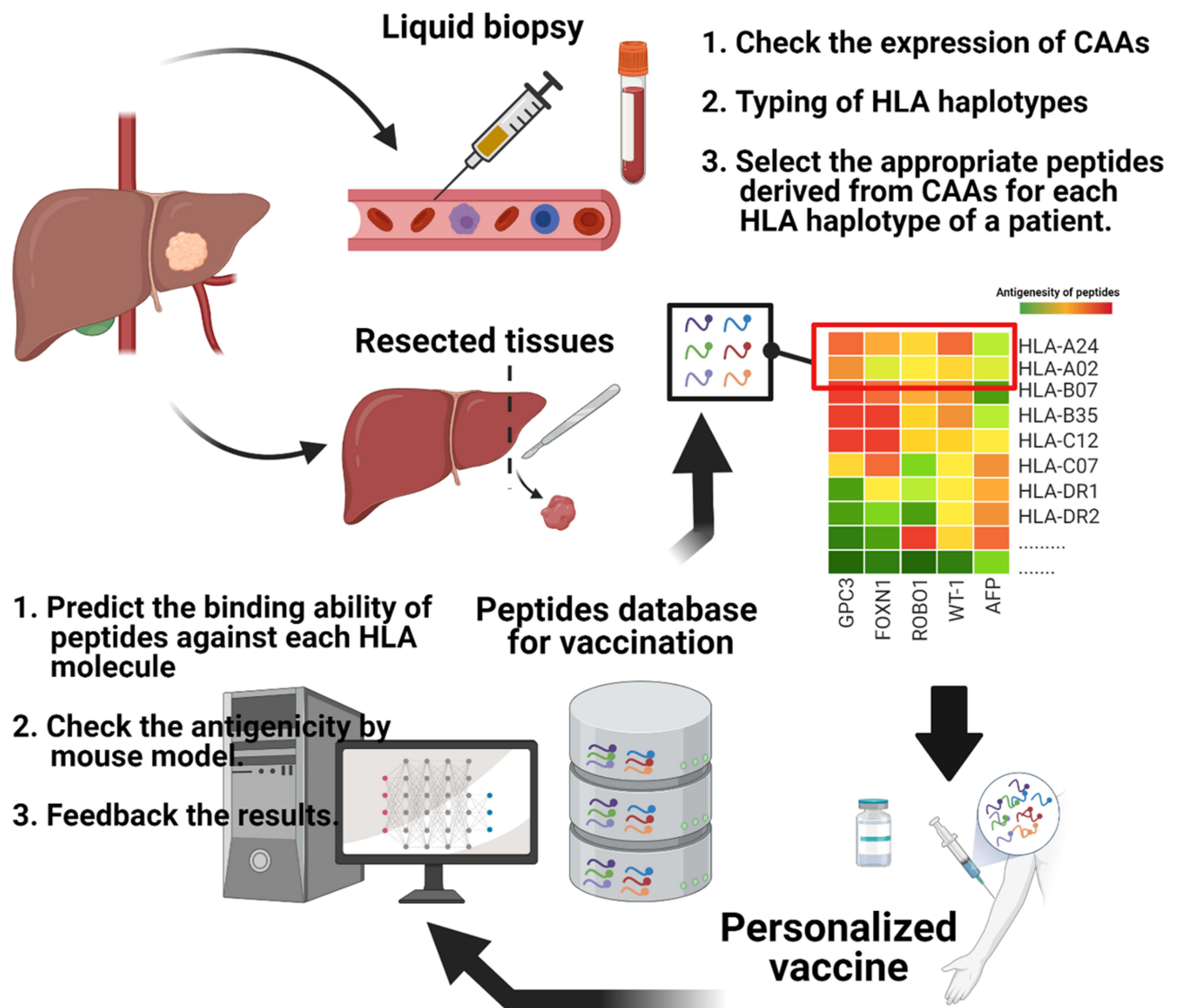


Figure 3 Scheme of personalized vaccination strategy targeting cancer associated antigens (CAAs). Personalized vaccines can be realized using a peptide prediction system for vaccines based on an off-the-shelf database of peptides derived from various types of CAAs.

Personalized Vaccine Targeting Neoantigens

“Neoantigens” are tumor-specific antigens that appear as a result of novel mutations in tumor cells.^{81,82} These proteins are degraded by cells with the ubiquitin-proteasome system, resulting in the production and release of several peptides, including those that present the novel non-synonymous mutation, which are termed “neoantigens”. Tumors with high tumor mutational burden (TMB) present a higher number of neoantigens. However, HCC usually has low TMB,⁸³ and hence, a deep analysis of the potential mutations and the development of personalized treatment

for each individual should be performed to develop vaccines based on neoantigens, instead of solely relying on TMB.^{84–86} Furthermore, a deep understanding of the underlying mechanism is needed, as the neoepitope generated might not correspond to the HLA of the patient, HLA allele-specific expression loss frequently occurs in tumors, and only some neoepitopes that will successfully be presented on the MHC may trigger specific T-cells if there is no strong immunosuppression.^{87,88} What kind of neoantigen is the best for developing a potential peptide vaccine in HCC that is able to induce immune memory still needs to be determined.^{89,90}

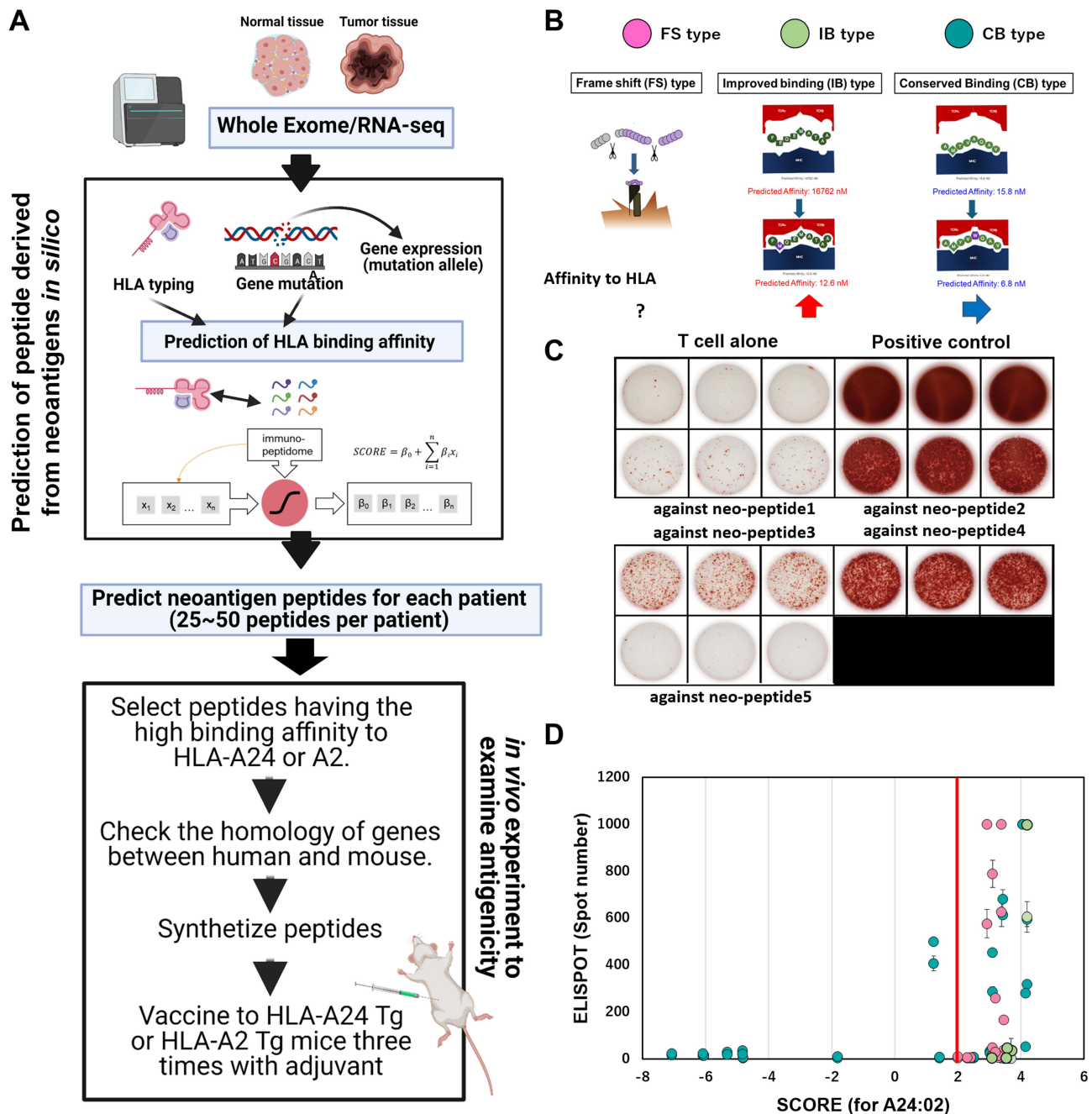


Figure 4 Scheme for the development of personalized neoantigen vaccines at the National Cancer Center. **(A)** In silico neoantigen prediction algorithm and its antigenicity validation DNA and RNA were extracted from the patient's tumor tissue, and whole exon analysis and RNA-seq analysis were performed using a next-generation sequencer. From the omics analysis of gene mutations and their expression in each patient, neoantigen-derived peptides that can bind to the patient's HLA were predicted in silico. We developed our own prediction algorithm in collaboration with BrightPath Biotechnology, Inc. A total of 25–50 predicted peptides were synthesized according to the prediction score per patient. For experiments using human HLA-transgenic mice (human HLA-Tg) experiments, peptides with wild-type sequences homologous to mice were selected and used for peptide vaccination with polyI:C:LC as an adjuvant. After repeated vaccination with human HLA-Tg, spleen cells were collected, and immune responses to the peptides were evaluated using IFN- γ -ELISPOT assay. **(B)** Classification of predicted neoantigen peptides by HLA binding affinity. Mutation-derived peptides were classified into three groups: frame shift (FS) type, improved binding (IB) type, and conserved binding (CB) type, based on the change in HLA-binding affinity depending on the location of the mutation in the peptide. **(C)** Validation of neoantigen peptide antigenicity using human HLA-Tg. Strong induction of immunity to neoantigens 02, 03, and 04 was confirmed. **(D)** Association between antigenicity prediction scores and immune responses to peptides. **(D)** Correlation between immunogenicity in HLA-A24-Tg and the prediction score of our algorithms in HLA-A24. Each dot shows a peptide that was predicted and assessed for its immunogenicity in vivo. The color of the dots shows the neoantigen types in **(B)**; red is FS type, blue is IB type, and green is CB type.

To evaluate the immunogenicity of neopeptides derived from gene mutations in HCC, we examined whether neopeptides could induce significant immune responses in vivo by

repeatable vaccination of transgenic (Tg) mice expressing human HLA. Whole exome sequencing (WES) of resected HCC tissues in patients who have HLA-A24 or A02 and

HLA typing were performed to find somatic mutations that occurred in the tumor cells. Using our original *in silico* prediction algorithm, neoantigens were predicted from non-synonymous somatic mutations and frameshift mutations and subsequently synthesized. Then, peptides derived from genes conserved between mice and humans were selected and used for vaccination of HLA-A24 or A2-Tg mice using poly-ICLC as an adjuvant for a total of three times. Until now we used IFA for peptide vaccines, but it was a poor immunological adjuvant, this is why we used poly-ICLC, and in parallel, we are checking which other adjuvants would be the best (CpG, mRNA ...). Splenocytes were harvested from vaccinated mice, and the response of T cells to predicted peptides was assayed by IFN- γ ELISPOT assay (Figure 4A and B). Positive responses to peptide injection were observed in 43% of the A24-Tg mice, and in 41% of the A02-Tg mice. These responses were mutation-specific and did not occur against the wild-type peptide (Figure 4C). These results indicate the immunogenicity of the neoantigen epitope. Our prediction pipeline was shown to be generally accurate and capable of extracting antigenic neoantigen peptides, since many or the peptides that induced an immune response were selected by the algorithm (Figure 4D). However, some of the HLA-A02 peptides showed immune activity, including peptides with low scores predicted by the algorithm. It is necessary to improve the prediction accuracy of the algorithm by integrating new information and results from further analyses. Our results demonstrated the availability of HLA-Tg mouse models to validate prediction algorithms based on physiological immune response *in vivo*. Moreover, these vaccination models also are useful for the check any adverse effects and off target effect by vaccination. Now, we are preparing for publication of these results.

To date, various clinical trials of cancer vaccines targeting neoantigens have been conducted, mainly by venture companies (Table 5). A vaccine with ICIs and peptides derived from DNAJB1-PRKACA fusion kinase is currently being tested for fibrolamellar hepatocellular carcinoma in Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (NCT04248569). However, since the majority of these trials are still in Phase I, the therapeutic efficacy still remains to be determined. Some studies have reported the induction of CTL and helper T cells in patients responding to the administered neoantigen vaccines. The final goal of our study was to develop adjuvant vaccines targeting TSAs to prevent early recurrence and metastasis, resulting in a better prognosis for HCC.

Summary

In summary, this review discusses several examples of positive results using peptide vaccines as a treatment for cancer. The induction of CTL in humans is promising. Furthermore, the technique is simple. We also described several common cancer antigens in HCC, which have been targeted in cancer treatment with peptides. Moreover, we discussed the potential for not focusing only on one cancer antigen or one peptide. Using previously reported studies, we concluded that some peptide vaccines against HCC antigens are efficacious and lead to potential tumor regression. Hence, there is a strong interest in peptide vaccine immunotherapy using a mix of common cancer antigens containing numerous and various epitopes for both HLA class I and II. However, the achieved efficacy is still partial, and so far, no total and definitive recovery has been observed. Cancers are complex from a physical molecular diversity perspective, with several mutations and adaptive mechanisms, challenging the innovation with the immunotherapies used. Advantages of TAAs-based vaccines are the density of peptides that are presented on HLA molecules by direct binding, the cost-effectiveness, the use of a peptide or a mix of peptides relevant for an immune response decreases the risk of contamination observed for inactivated or attenuated vaccines and reduces problems linked to autoimmunity and immune evasion. However, disadvantages are the necessity to use peptides having high immunogenicity, hence the interest of a higher *in silico* prediction or peptidome analysis, and the HLA haplotypes diversity which remains a strong challenge to design tailor-made vaccines with peptides against multiple antigens (including neoantigens). The need for strong antigens has led to intensive research on neoantigens specific to cancers, which are thought to be more immunogenic, leading to enhanced immunological responses. The use of strong adjuvants is also required for peptide vaccines. We have tried to use peptide vaccines that prevent recurrence as adjuvant therapy. For treatment of progressive cancers, peptide vaccine alone is not enough to induce regression, so it would require a peptide vaccine combined with powerful therapy such as ICI or radiotherapy. Our clinical trial suggests that the use of peptide vaccine as adjuvant therapy after surgery is good to prolong overall survival, especially for hepatoblastoma patients. Vaccine are usually used to prevent infections, so we want to use cancer vaccine to prevent recurrence or

Table 5 The Development of Personalized Vaccines Targeting Neoantigens

Sponsors/Locations		Cancer Type	Vaccines
University of Pennsylvania	USA	Melanoma	Peptide-treated DC
Washington University	USA	Glioblastoma	Peptide, poly-ICLC
Immatics, BioNtech AG	Europe	Glioblastoma	Peptide, poly-ICLC, GM-CSF
Agenus Inc.	USA	Advanced tumor	Peptide, QS-21, HSP70
National Cancer Institute	USA	Solid cancer	Polyepitope RNA
Radboud University	Europe	Colon cancer	Frame shift-derived peptide-loaded DC
National Cancer Institute (NCI)	USA	Breast cancer	Long Peptide Vaccine, Poly ICLC
TRON, BioNtech AG	Europe	Melanoma	Polyepitope RNA
Dana-Farber Cancer Institute	USA	Melanoma	Peptide, poly-ICLC
Washington University	USA	Breast cancer (TN)	Polyepitope DNA
Icahn School of Medicine at Mount Sinai, Genentech, Inc.	USA	Urothelial cancer Bladder cancer	Peptide, poly-ICLC, Atezolizumab
TRON, BioNtech AG	Europe	Breast cancer (TN)	Polyepitope RNA
Icahn School of Medicine at Mount Sinai	USA	Solid cancer	Peptide, poly-ICLC
Washington University	USA	Breast cancer (TN)	Peptide, poly-ICLC
Advaxis, Inc. Amgen	USA	Colon cancer, Head and Neck cancer, NSCLC	Peptide
Genentech, Inc. Biontech RNA Pharmaceuticals GmbH	USA	Solid cancer	Polyepitope RNA, Atezolizumab
Neon Therapeutics, Bristol-Myere Squibb	USA	Lung cancer Melanoma Bladder cancer	Peptide, poly-ICLC, Nivolumab
Neon Therapeutics, Inc. Merck Sharp and Dohme Corp.	USA	NSCLC	Peptide, poly-ICLC, Pembrolizumab
Washington University MedImmune LLC	USA	Breast cancer (TN)	Polyepitope DNA, electroporation
Icahn School of Medicine at Mount Sinai NovoCure Ltd.	USA	Glioblastoma	Peptide, poly-ICLC, Tumor Treating Fields
M.D. Anderson Cancer Center	USA	PDAC Colon cancer	Peptide, IFA
Dana-Farber Cancer Institute	USA	Glioblastoma	Peptide, poly-ICLC, Radiotherapy
Washington University	USA	PDAC	Polyepitope DNA, electroporation
Washington University School of Medicine, Children's Discovery Institute	USA	Brain tumor	Personalized neoantigen DNA vaccine

(Continued)

Table 5 (Continued).

Sponsors/Locations		Cancer Type	Vaccines
Dana-Farber Cancer Institute, Bristol-Myere Squibb	USA	Renal cancer	Peptide, poly-ICLC, Ipilimumab
Washington University School of Medicine, National Institutes of Health (NIH), National Cancer Institute (NCI)	USA	PDAC	Neoantigen Peptide Vaccine, Poly ICLC
Washington University School of Medicine, MedImmune LLC	USA	Renal cancer	Neoantigen DNA Vaccine
Dana-Farber Cancer Institute Oncovir, Inc. Neon Therapeutics, Inc.	USA	Lymphoma	Peptide, poly-ICLC, Cyclophosphamide
Washington University School of Medicine Bristol-Myers Squibb Prostate Cancer Foundation BJH Foundation	USA	Prostate cancer (metastasis)	Neoantigen DNA vaccine, TriGrid Delivery System
Washington University	USA	Glioblastoma	Peptide, poly-ICLC, Ipilimumab, Nivolumab
Washington University School of Medicine	USA	Brain tumor	Personalized neoantigen DNA vaccine
Washington University	USA	Brain tumor	Peptide, poly-ICLC
Washington University School of Medicine	USA	Brain tumor	Personalized peptide vaccine, Poly ICLC
Dana-Farber Cancer Institute	USA	Follicular lymphoma	Peptide, poly-ICLC
Washington University	USA	Follicular lymphoma	Peptide, poly-ICLC, Nivolumab

Notes: No color = used peptide for vaccination, Gray fill = synthesis of nucleotides used for vaccination (DNA or RNA). As of November 2019.

de novo tumor for patient with high risk factors (for HCC or other cancers). However, these points still need deeper investigation. The main aspect remaining to be resolved for the development of successful peptide vaccines is identifying which targets are the most effective. As previously mentioned, several antigens are overexpressed and mutated, but not all lead to a complete response. One reason why these peptide vaccines failed to induce tumor regression, despite the appearance of CTL by vaccination, may be that the CAA-derived peptides used for vaccination were not presented to HLA molecules on the tumor cell surface. HLA-ligandome analysis based on the identification of amino acid residues by mass spectrometry is an interesting, attractive and important approach to identified peptides that are presented on HLA therefore may be recognized by CTLs during tumor rejection. However, proteomics analysis has issues, only some

peptides might likely be ionized during mass spectrometry analysis so it is difficult to find every peptide that may be presented on HLA. We used HLA ligandome analysis to determine neo-epitopes, but it is not enough and new techniques are required.⁹¹ Concerning neoantigen-based immunotherapy, challenges are the still low power prediction of algorithms to efficiently predict immunogenic peptide-derived neoantigens. Also, the poor research and prediction for CD4⁺ T cells needs research. However, concerning low frequency neoantigens in low mutational burden tumors, recent study demonstrated the use of radiotherapy to induce mutation, therefore neoantigens.⁹² Neoantigens belong to the intra-tumor heterogeneity, therefore, it is necessary to identify all neoantigens present in tumors to get an efficient treatment. These issues need further investigation in the future.

Data Sharing Statement

The results that support the findings of Figure 4 are not publicly available due to our preliminary data. Now, we are going to submission for any public journal.

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Disclosure

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