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Review Article

Recent progress on MHC-I epitope prediction in tumor immunotherapy

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Abstract: Tumor immunotherapy has now become one of the most potential therapy for those intractable cancer diseases. The antigens on the cancer cell surfaces are the keys for the immune system to recognize and eliminate them. As reported, the immunogenicity of the tumor antigens could be determined by the binding between the key epitope peptides and MHC molecules. In recent years, the approaches to anticipate the peptides from the candidate epitopes have gradually changed into more efficient methods. Including the improved conventional methods, more diverse methods were coming into view. Here we review the anticipated methods of the tumor associated epitopes that specifically bind with major histocompatibility complex (MHC) class I molecules, and the recent advances and applications of those epitope prediction methods.

Keywords: CTL epitopes, MHC class I molecule, prediction and identification, tumor immunotherapy, epitope prediction

Background

Nowadays, malignant tumors are the main death causes of human beings. The low cure or survival rate and the high morbidity of cancer result in millions of new cases and deaths of cancer each year [1]. The poor prognoses of the cancer were mainly caused by immune escapes. As a reason, immunotherapy based on specific immunity has now become another promising strategy for cancer treatment, after surgical treatment, radiotherapy and chemotherapy [2]. Based on the development of immunology, relying on the active immune system has become a leading option to overcome the intractable cancer problems [3]. Tumor immunotherapy is characterized by stimulating the specific immune response to enhance the immune rejection to inhibit and kill the tumor cells, thus reducing the ability of tumor recurrence and metastasis. In immunotherapy, the antigens on the cell surfaces are the keys for the immune system to distinguish normal cells from the cancer cells.

As an effective therapeutic form of immunology, the treatment against cancer via peptide vaccinations has been quite mature during the last decades since first reported in 1990 [4], that aiming to induce the anticancer immunity based on the identified antigen [5]. The active immune process induced by antigen would be initiated by antigen presenting cells (APC) (mostly dendritic cells) phagocysis the peptides. APCs then cross-presentate the antigen peptides to the CD8+ T cells by MHC class I molecules: These peptides were collectively called MHC-I ligands, which are usually 8-11 amino acids. The recognition of peptide-MHC complexes by CD8+ T cells establishes the antigenicity of the peptide [6]. They would be activated by peptides specifically and eliminate the targeted tumor cells via generation and stimulation of cytotoxic CD8+ T lymphocytes (CTLs) [7].

Antigens and epitopes

Epitopes are the cognate antigens of the T-cells recognize portions. Identifying the epitopes in

antigens helps researchers to anticipate the related possible immune based therapy. Large amounts of the studies focus on the T-cell epitope prediction to identify the shortest peptides within the antigens that are able to stimulate T lymphocytes such as CTLs. The peptide would determine the final immunogenicity which specifically depends on antigen processing, peptide-MHC binding and cognate T cell receptor (TCR) recognition. The key of the peptide vaccine delivery treatment was the consisting and optimizing of these peptides that derived from the candidate epitopes such as tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). TAAs are antigens in tumor cells that are expressed from overexpressed genes in tumors. While the TSAs are antigens restricted to tumor cells, including those neoantigens that arise from DNA mutations. The vast majority immunotherapies so far focus on tumor antigens (TAs), such as melanoma differentiation antigen MART-1 [8]. However, with little T cells activated effects, targeting TA therapy had not shown significant success in the clinical trials.

Most of the TAs including epitopes described above required proteasome activity: generally, before being loaded on the MHC-1 molecules, these folded proteins are degraded by proteasomes and transferred into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP) to become peptide-MHC (pMHC) complex. Stable peptides are then presented to the cell surface via the MHC-1 complex and can be identified by monitoring CD8+ T cells. Yet the defects of the proteasome-TAP-mediated peptide-processing pathway is also a key reason of the immune escape. For instance, T-cell epitopes associated with impaired peptide processing (TEIPP) antigens are antigens with an alternative antigen-processing route [9]. These antigens could bind with MHC-I while the APCs are lacking of TAP, right after undergoing the intramembrane proteolysis by the signal peptide peptidase (SPP) [10]. Former researchers even developed neoepitopes derived from the preprocalcitonin (ppCT) leader sequence that to be processed independently from conventional proteasomes-TAP route [11] (**Figure 1**).

In order to induce the T-cell immunity better, the targeted modified peptides must be seeking that can be presented to MHC molecules to improve the therapy efficacy. As the researchers have paid more attention to the epitopes nowadays, different methods for predicting peptide binding to MHC molecules are developing rapidly.

Recent discoveries of the MHC-I epitope peptide screening process

A decade before, effective epitopes peptides were preferred to be predicted and screened from combinatorial peptide libraries, or from the phage display via the epitope libraries (made of phage vast clones) [12, 13]. These methods for screening cost less and use simple, however, could not ensure all the peptides are immunogenic high affinity or specific. As a reason, these approaches have gradually changed into more advanced ones. More efficient methods like immunopeptidomes, genomics and bioinformatics have been widely used nowadays that can help us to predict and identify the TSAs, MHC ligands as well as the corresponding epitope peptide more rapidly and accurately.

MHC-I epitope in silico prediction

MHC-I molecules, termed as Human Leukocyte Antigens (HLA) in humans, are particularly polymorphic in the human population which have highly dependent peptide bounds with individual's HLA allotypes.

In silico methods largely improved the sensitivity of the MHC I-bound peptide prediction. Different algorithms have been developed for predicting the binding affinity between peptide and MHC-I molecules. As reported before [14], MHC ligands of equal length can be naturally aligned and via identifying the specific sequence motifs, models of binding affinity could be predicted and trained throughout the known ligands for the given MHC I alleles. A number of new computational tools have been developed for predicting peptide binding to HLA-I molecules due to the increasing availability of high-quality HLA allele-specific data sets in recent years.

Most of the experimentally verified HLA ligand sequences are deposited in public peptide ligand databases. The Immune Epitope Database (IEDB) is one of the largest public resources for HLA ligand and T-cell epitopes [15], which includes a number of prediction techniques.

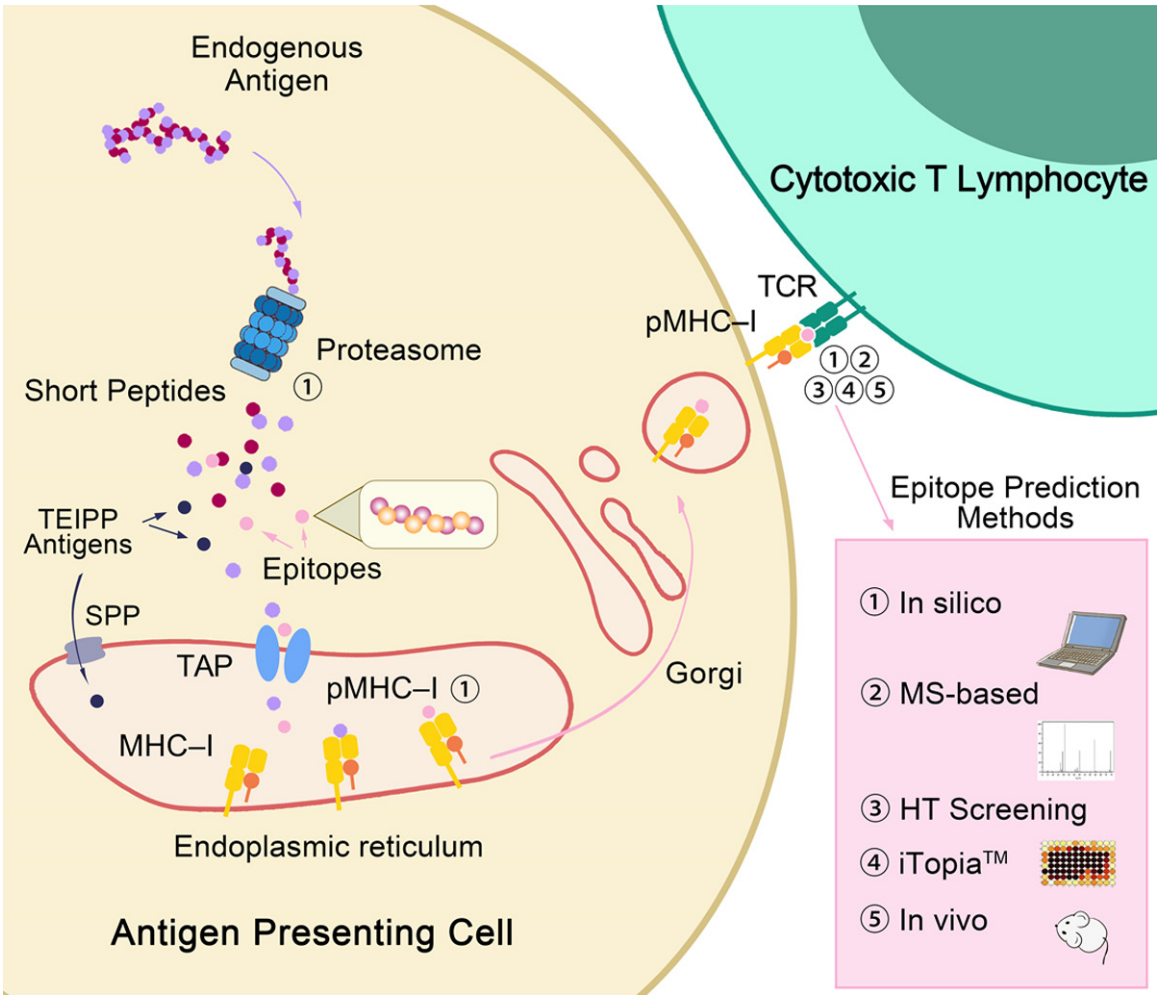

Figure 1. The MHC Class I epitope transfer processing. The left part represents the major processes of the cognate antigens-epitopes inside the APCs and are presented by MHC I molecules to the surface of the APCs. Endogenous antigens are digested by the proteasome, most of the peptide fragments were then transferred into the ER by the TAP and form pMHC complex with MHC-I molecules. While TEIPP can only pass the ER by intramembrane proteolysis while the APCs lack of TAP, with the help of signal peptide peptidase (SPP). After pMHC complexes formed, they would generate the elimination process via stimulating the CTLs. According to the current mainstream MHC ligands prediction methods, here we classify the prediction methods mainly into four aspects: 1. In silico methods: including sequence-scoring based methods; 2. Machine learning based methods (artificial intelligence), predictive tools are available throughout the whole epitope antigen presentation process; immunoaffinity predictions: mainly based on mass spectrometry (MS), including different proteomics strategies; 3. High-throughput screening (HTS) based methods; 4. iTopia epitope discovery system; 5. In vivo series assays.

Formers classified the present computational tools into mainly three categories based on their methodologies: sequence-scoring based methods, machine learning based methods (artificial intelligence), and some other structure-based methods [16].

Sequence-scoring: The first category of in silico methods is developed based on different statistical scoring functions: they score the alternative peptide sequences via calculating the sequence similarity, amino acid frequencies, position-specific and such other certain features to generate the motifs of specific HLA alleles. After the position-specific scoring matrix (PSSM) was first generated [17], it has been widely used for specifying the information of amino acids at different positions of a peptide sequence [18], which allows us to calculate the score of a peptide via multiplying the frequencies of its corresponding amino acids at each position.

Table 1. The summary of mentioned in silico tools

Category	Tool name	Invented (Year)	Website	Algorithm	Available	Latest Updating
MHC Based						
Scoring function-based	SYFPEITHI	1999	http://www.syfpeithi.de/	PSSM	Yes	2012.8.7
	RANKPEP	2002	http://imed.med.ucm.es/Tools/rankpep.html	PSSM	Yes	2009.6.3
	BIMAS	1994	http://bimas.dcrt.nih.gov/molbio/hla_bind	HLA-BIND	No	-
	MixMHCpred 2.1	2017	https://github.com/GfellerLab/MixMHCpred	PSSM	Yes	2020.4
	PromPDD	2019	http://www.immunoinformatics.net/PromPDD/	ARBM	Yes	-
Machine learning-based	NetMHC 4.0	2016	http://www.cbs.dtu.dk/services/NetMHC-4.0	NN	Yes	-
	NetMHCpam 4.1	2017	http://www.cbs.dtu.dk/services/NetMHCpan-4.1/	NN	Yes	2019.12
	ProPred1	2003	http://crdd.osdd.net/raghava/propred1/	QM	Yes	-
	SVMHC	2002	http://www.sbc.su.se/%7Epierre/svmhc/	SVM	No	-
	SVRMHC	2006	http://SVRMHC.umn.edu/SVRMHCdb	SVR	No	-
	ForestMHC	2018	https://github.com/kmboehm/ForestMHC	NN	Yes	-
Conesus method	MHCcombine	2019	http://mhccombine.dkfz.de	multiple	Yes	-
MS-based						
Proteomics	MaxQuant		https://www.maxquant.org/	MaxQuant	Yes	2019.2
	SysteMHC Atlas	2015	https://systemhcatlas.org/	-	Yes	2017.8

PSSM: position-specific scoring matrix; ABRM: average relative binding matrix; NN: neural network; ANN: Artificial neural network; QM: Quantitative Matrix; SVM: support vector machine; SVR: support vector regression.

Among them, SYFPEITHI and RANKPEP are two of the most reliable and widely used tools. SYFPEITHI [19] calculates the score by adding the corresponding value of each amino acid at each position, while RANKPEP [20] uses the motif profiles from calculating the PSSM of ligands bound to the given HLA allotypes. In addition, the conventional bioinformatics and molecular analysis section (BIMAS) is continue being used: it was first created by Parker KC et al. in 1994 to establish the total peptide binding motif of the human MHC molecule HLA-A*0201 and to develop a complete matrix [21], which can still successfully screened epitope peptides efficiently nowadays [22]. Comparing of these latest provided scoring function-based tools, one tool named MixMHCpred 2.0.1 [23], achieved the best performance because of its highest AUC (area under the receiver operating characteristic curve) values among nearly all 19 HLA allotypes, due to its latest public HLA-peptide data sources, according to the Mei S. et al. [24]. It was updated to version 2.1 at 2020 April and has expanded HLA-I allele coverage. In addition, the scoring-based tools with other algorithms were coming into view: PromPDD that based on the average relative binding (ARB) matrix (proposed in 1993 [25]) was designed for deciphering and designing of promiscuous peptides bind to HLA-I molecules [26].

Yet even these widely used algorithms may have defects: the present accuracy of the affinity between the predicted peptide and HLA molecules is still not satisfying. As being designed to predict the binding affinity, these traditional algorithms would not account for intracellular availability of the peptide precursors or their processing by proteases. Maria Bonsack et al. evaluated most of the prediction tools and reported that only 151 of 242 (62%) positive predictions matched with actual binding, while 127 of 278 (46%) true binders were even not predicted within the given thresholds after analyzing all HLA types [27]. To distinguish peptide bindings accurately, suitable prediction algorithms shall be chosen depending on HLA type and peptide length, because not all peptides that were predicted to be binders in silico bound experimentally. In their study, Maria et al. provided the better tolerant thresholds for those former predictions to increase the sensitivity, and developed a web application: MHC combine, to facilitate the simultaneous use and comparison of multiple MHC class-I peptide prediction algorithms (**Table 1**).

Machine learning: Since the scoring-based approaches only handle linear features of the peptide, various machine learning approaches now have been developed for HLA-peptide binding prediction. These machine-learning tools could identify the peptide whether a bind-

er or not of the HLA molecules by generating a score based on extracted feature (0 or 1) via constructing a training model.

Artificial neural network (ANN) was first applicated for cancer in 1991 [28], and developed for HLA prediction in 1995 [29]. In this approach, peptide sequences are transformed into numeric descriptors (scoring) then fed to several layers of artificial neurons, with a given value from the previous layer via mathematical formula to calculate the final prediction value. Some other machine learning tools such as SVMHC and SVRMHC, that are based on support vector machine (SVM) and regression (SVR) modeling peptide-MHC binding approaches, were popular before but haven't been updated for about ten more years, nor the website available [30, 31]. In contrast, the neural network was preferred utilized to construct the prediction models in a number of machine-learning-based tools nowadays, including NetMHC 4.0 [32] and NetMHCpan 4.1 [33], the two similar and most widely used algorithms.

Briefly, NetMHC mainly uses an ensemble method to generate the neural network and assigns the binding core (nine amino acids), some extra complementary sequence-based features such as length or compositions of the terminal regions also enable the algorithm to learn the complex binding patterns from the peptide-HLA-I molecule. The NetMHCpan is similar with NetMHC about the neural network constructing, yet the peptides it was trained by were generated from both binding affinity assays and those peptide ligands identified by mass spectrometry (MS) pairing. Besides, pseudosequences of HLA-binding pockets were also used in NetMHCpan to calculate the similarities of different HLA allotypes ligand bindings, that made this approach achieve a better prediction compared to the others. Lately Kevin et al. developed a promising method: ForestMHC that is also used to identify peptides bound by MHC-I [23]. Their random forest approach was trained by assembling the largest known database of MS binding data to show and confirm the effect of gene expression on peptide presentation, which was reported outperforms NetMHC and NetMHCpan on test sets. Furthermore, some other new developed algorithms that are specific to neoantigens, such as MHCSeqNet, INeo-Epp, are potential generalization to those unseen MHC class I alleles [34, 35] (**Figure 2**).

MHC-I epitope in vitro screening

Despite these in silico approaches mentioned before, there are also some in vitro approaches valued a lot, such as using the immunopeptidome pathways to predict and isolate the peptide. Numbers of the informatic data were generated by using in vitro binding assays. Among these, the employing of the MS based identification of purified MHC-binding peptides is always an essential component.

The method called acid elution (AE) was initially applied to eliminate the MHC-I molecules [36]. In 1993, acid elution was developed for isolating the CD8+ T-cell epitopes and β2-microglobulin from the peptide-MHC-I complexes: they acid-elute the immunogenic peptides from the cell-free supernatants and then separate them with reverse-phase high performance liquid chromatography (RP-HPLC) [37]. This approach is simple yet may bring damage to the peptide as the cells may release protease simultaneously [38]. Another well-known step called immunoprecipitation (IP) then shall be required. It applied the isolated MHC-I complexes to the columns and coupled with monoclonal antibodies. The peptides then would be then isolated from the HLA complex through the AE steps mentioned before and the IP purification.

The sequence of the peptide would then be analyzed by tandem mass spectrometry (MS/MS), with further in vitro and silico validation [39]. Mass spectrometry-based pathway for MHC-bound peptide prediction was pioneered by D. Hunt et al. in 1992 [40]. Its sensitivity and speed developed a lot through the decades. The available endogenously processed and presented ligands identification increased from dozens to thousands [41-43]. In the present, liquid chromatography-tandem mass spectrometry (LC-MS/MS) based immuno-peptidomics is still the most widely used method to identify and quantify the HLA peptides [44], through which the information of endogenously processed could be directly obtained. Recently, Anthony et al. developed an approach by using a nano-ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (nUPLC-MS/MS). The isolation quality for

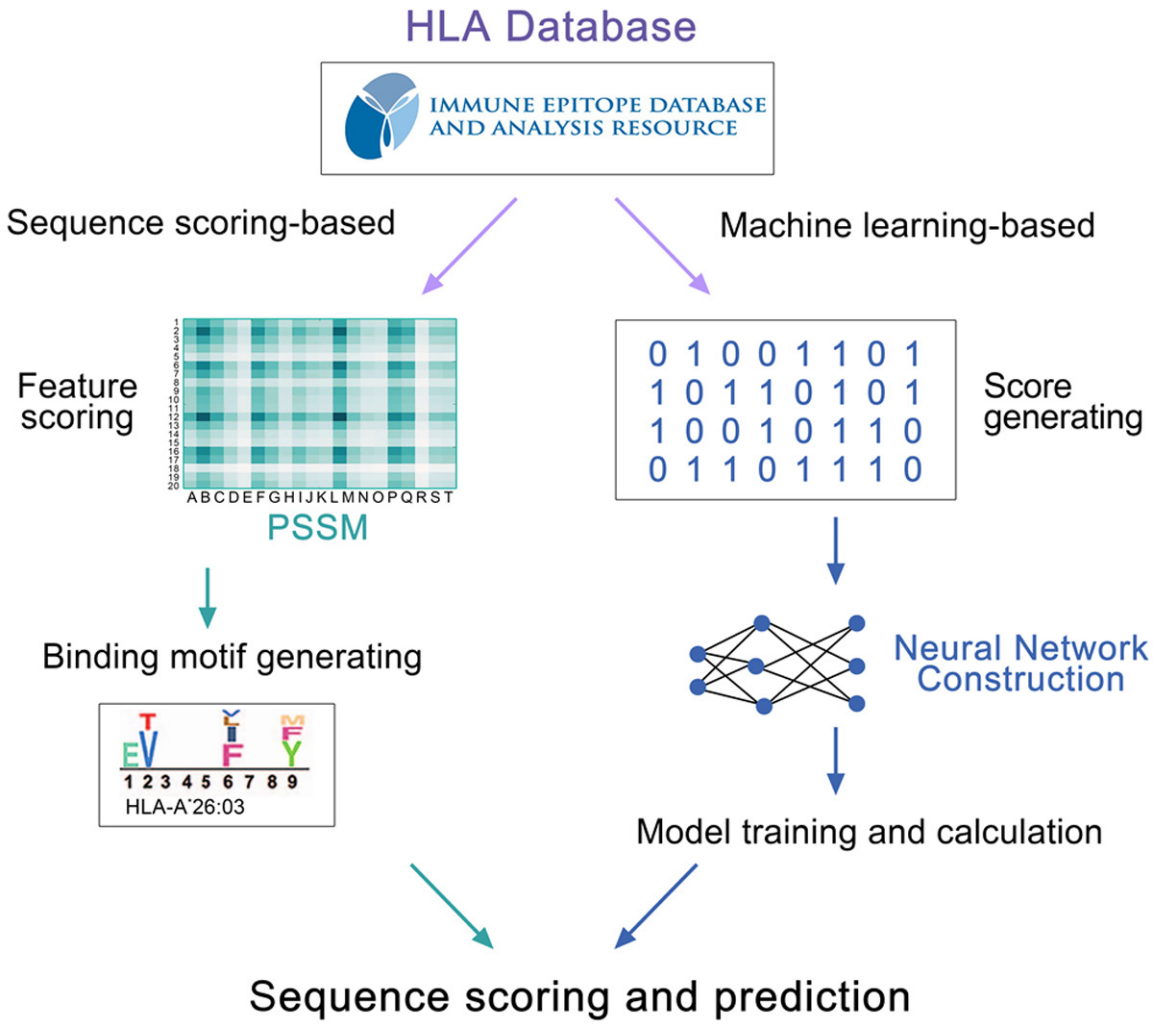

Figure 2. The process schematic of the two different machine learning prediction methods. Left: The sequence-scoring based method: after scoring the alternative peptide sequences via calculating the certain features of HLA alleles, the position-specific scoring matrix (PSSM) was generated, peptide binding motif would next be formed and allows to calculate the sequence score for prediction. Right: The machine-learning based method: identify a score (0 or 1) based on extracted feature (whether a binder or not of the HLA molecules) for peptide sequences, then fed to several layers of artificial neurons, trained and calculated the final prediction score with the given value from previous layer.

the peptides was improved via this reported new protocol [45].

To identify the MHC ligands from LC-MS/MS data, proteomics strategy: searching engine and protein database, were required. The approach was firstly employed in 1994 by J. Eng et al. to interpret tandem mass spectra with known sequences in a protein database via matching the spectra with the predictable protein fragments cleaved by proteolytic enzymes [46]. It has now widely developed into numbers of peptide-matching tools such as MaxQuant [47], ProteinPilot [48], Proteome Discoverer [49] and etc. that are based on different algorithms for peptide prediction [50], as well as relevant databases like SysteMHC Atlas [51]. As proteomics searching without an enzyme specificity, strict false discovery rate (FDR) estimation cut-off (about 1%) and filtration should be subsequently required to ensure the accuracy of the ligands assignment. Recently Andreatta M. et al. proposed an approach called MS-rescue which could be employed for rescoring peptide-spectrum matches that not confirming to the 1% FDR cut-off [52]. Some in silico tools were also developed to improve the identification performance because of the

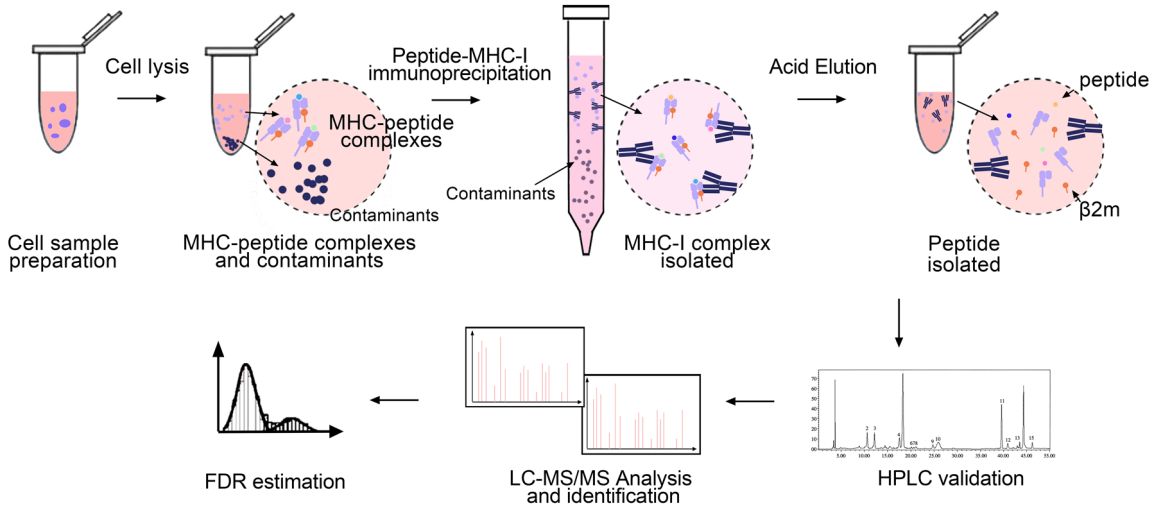

Figure 3. Main steps of MS-based identification: immunoprecipitation, acid elution, the later tandem mass spectrometry analysis and the in silico proteomics validation. The immunoprecipitation: the MHC-peptide complexes were isolated from the lysed samples first, with the monoclonal antibodies inside the wall of an isolating column, the MHC-I complexes were isolated then. Acid Elution: isolate the CD8+ T-cell epitopes and β2-microglobulin from the peptide-MHC-I complexes. The isolated immunogenic peptides would move to the further MS analysis and in silico validation.

Table 2. Epitope antigens that recently screened with combinatorial methods

Antigen	Approaches	Sequence	Reference
MUC1	SYFPEITHI, NetMHCpan 4.0 and IEDB	YKQGGFLGL	[97]
WT-1	SVMHC, RANKPEP, IEDB, MHCPEP and SYFPEITHI	RTPYSSDNLYQMTSQLECMTWNQMNL	[98]
HPV16	IEDB, ANN and ELISPOT	LTAPTGCIKK	[99]
HSP90	In vivo assays, MS and Proteome Discoverer	NVP-HSP990	[100]
HPV16	NetMHC 4.0, NetMHC 3.4, NetMHCcons 1.1, SMM, BIMAS and SYFPEITHI	RTLEDLLMGT	[101]
CTA	MaxQuant, μLC-MS-MS and NetMHC	EADPTGHSY	[102]
XAGE-1b	NetMHCpan 2.8, IEDB, ANN, SMM and Comblib	SPKKKNQQL	[103]
HPV L1&L2	NetMHCpan 4.0, SYFPEITHI, ProPred1 [104] and in vivo assays	EATVYLPPVPVSKVV	[105]

insufficient protein information: SpectMHC was proposed based on the NetMHC predictions strategy to compile peptide databases for searching MHC-I IP MS data: Instead of matching the entire protein database with the peptide spectra, it constructed the database based on the priori-predicted potential MHC ligands, yet this approach may fail on those poorly characterized MHC alleles' motifs [53] (**Figure 3**).

Combined utilization

A variety of methods have been enumerated though, with the comprehensive use of the multiple approaches, the accuracy of the prediction, screening and identification of epitopes could be improved further. The **Table 2** concluded some recent MHC-I epitopes that mainly screened via multiple strategies (**Table 2**).

Other categories of MHC-I epitope anticipate method

Other than the two main categories methods mentioned above, some other functional tools could also efficiently screen the epitopes.

High-throughput screening methods: The TCR epitope scanning method called T-Scan was invited in 2019 by Tomasz Kula et., which is a high-throughput and genome-wide platform to identify the cognate antigens of T cells via using a 'cell-based pooled' screen [54]. In this assay, the T cells were firstly co-cultivated with a lentiviral candidate antigen library, the targeted cells with the toxic granules were isolated with a fluorescence-activated cell sorting (FACS) next, and finally the PCR and next-generation sequencing (NGS) were used to identify the

antigens specifically. During FACS process, T cell recognizing would be carried out by delivering a kind of granzyme B. It was also employed in another high-throughput screening assay with the same usage that to initiate and trace the apoptotic cascade that leads to cell killing. The another assay was then characterized by sequencing the encoded minigenes after the FACS part which was followed by validation with high-complexity, epitope-encoding minigene libraries [55].

Because of the recent publication, no tumor epitope screening has been carried out so far via the potential high-throughput screening except for the epitopes mentioned in the two articles that discovered these assays. However, compared with the predictive algorithms, the high-throughput screening methods rely on not only the binding affinity between the T cell receptors and the peptide-MHC complexes, but also the killing activity of the T cells, which is way better than the algorithms, since the paired sequences and these libraries would help to design T cell-based immunotherapeutic better.

Biochemical method: iTopia™: iTopia is a high-throughput biochemical technology to identify the CTL epitopes that enables the peptides to be evaluated and ranked rapidly based on their bindings, affinity and stability with the HLA class I molecules via an anti-HLA antibody [56]. It was reported had employed to identify the epitopes in the human TAA 5T4 successfully [57]. The combination use of iTopia methodology with the in silico tools has been reported to improve the accuracy and the efficiency of the epitope identification, compared with using one single approach: Nectin-4 (another kind of TAA) was recently screened via the iTopia Epitope Discovery System™ with additional use of the in silico tools: SYPEITHI and BIMAS [58] (**Table 1**).

The only problem of the iTopia is the bias: the number of peptide binders were likely to bias towards HLA-A*0201 with the lowest number of A*0101 in different studies respectively, with unclear reason [56, 57].

Series binding assay for peptide identification: Though various in silico and in vitro methods were mentioned for the peptide screening, their efficacy is still hard to guarantee because of the complex mechanism composition of the antigen presentation. To verify the actual binding effect of the predicted peptides, a series of binding assays could be employed. ELISPOT is widely used to observe the promotion of peptide-specific immune responses in peripheral blood mononuclear cells in recent years [59], which was also reported can be used to identify MHC-I epitopes [60]. Systematic peptide libraries were used in 96-well plates and the shorter peptides were preferred because of the both closing ends of the MHC-I peptide-binding groove [61]. The peptides competition for MHC binding and the peptide toxicity in this assay, however, would substantially limit the accuracy of the results [62].

In fact, after the algorithm predictions, binding assays including IFN-γ ELISpot restimulation, pMHC tetramer staining [63], TAP-deficient T2 lymphoma cell line testing and in vivo model (HLA-transgenic animals to represent human MHC-I alleles) testing were sequentially used to screen the immunogenic peptides with ideal binding affinity. To define the epitope peptide, some of the derived immunogenic peptides of TAAs such as MUC1 [64], hPEBP4 [65], CUE-101 [66], DKK1 [67] et al. were all separately characterized and screened via those comprehensive assays mentioned.

Optimizing modification of the predicted peptides

Based on the existing screening methods, a series of optimizations of the tumor epitope peptides could take place for the better practical application, including self-based structural modification and the extra use of other auxiliary materials (**Figure 4**).

Peptide altering

Nowadays, as the mysteries of the interactions between peptide and MHC-I molecules are being exposed, people gradually drive to the individual peptide modifications to increase the stability and immunogenicity of the pMHCs via optimizing the interactions between peptide anchor residues and MHC binding pockets. Peptides alterations were proved can enhance the presentation of the MHC-I epitope, that including peptide N-glycosylation [68], peptide shortening optimization [69] and the classic peptide substitution [70, 71], recently Abraham Sachs et al. even use α-aminobutyric acid

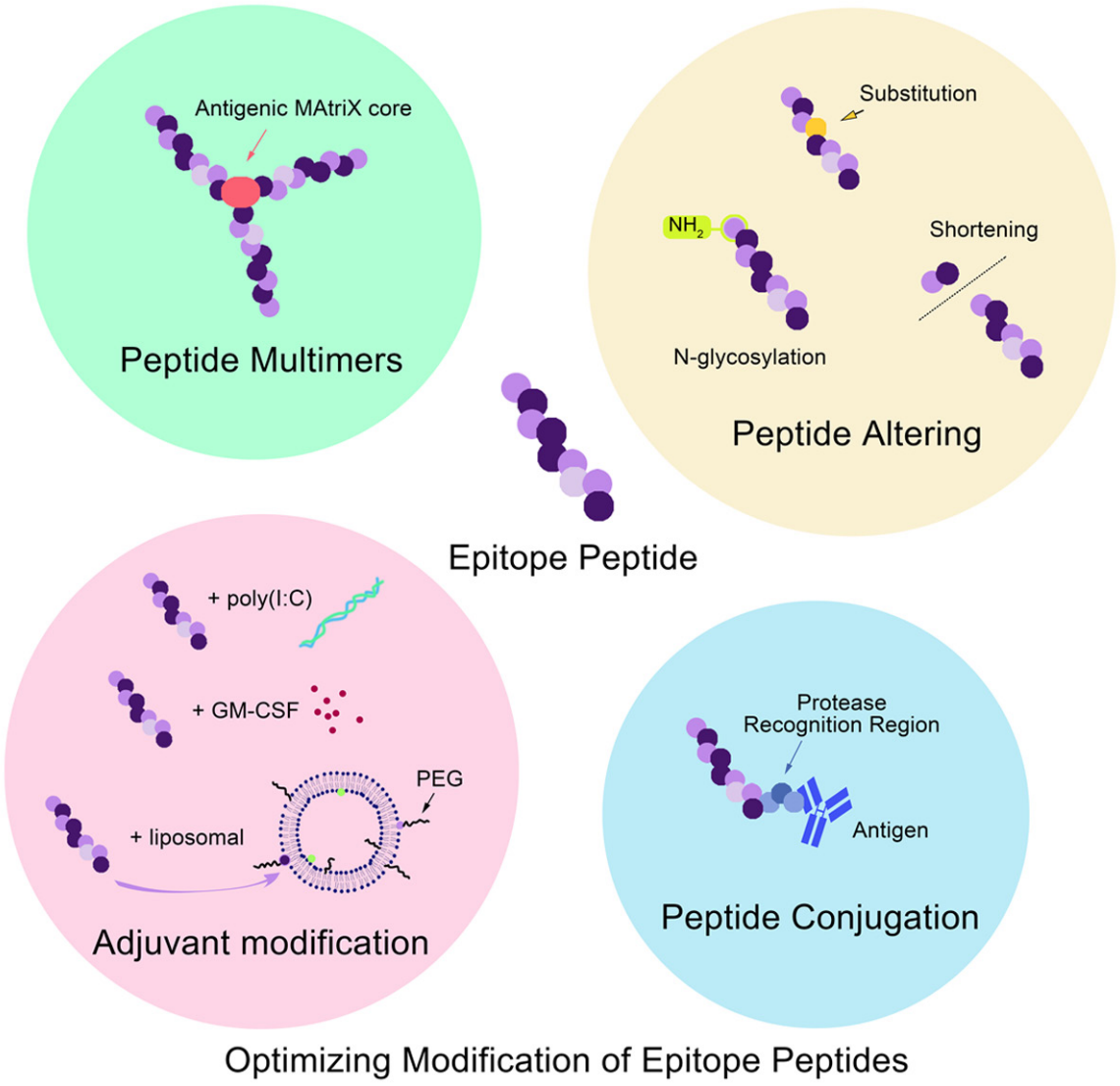

Figure 4. Optimizing modifications of epitope peptides.

(AABA) instead of amino acid to substitute the cysteine that to increase the assessment of immunoaffinity of cysteine-containing peptides [72]. Some position-specific alteration such as the position 3 proline-altered (p3P) optimization was reported that successfully increased pMHC complex stability [73], a relevant anti-cancer vaccination with a p3P altered TEIPP neoepitope Trh4 was also reported an enhancive immunogenicity.

Peptide conjugation

Conjugate modification is another rising anti-cancer approach. Peptide-drug conjugates (PDCs) are widely used as prodrugs for targeted drug delivery and chemotherapy in cancer treatment. With special linkers, drugs and antibodies can be conjugated with proteins and peptides, and active the targeted receptors on the tumor cells for more effective treatment [74, 75]. Similar modifications were used on epitopes: Antibody-peptide epitope conjugates (APECs) were made by the conjugation of the tumor-targeting antibodies and the protease-sensitive linkers. The epitopes could be derived from ovalbumin [76], cancer testis antigen [77] or even viruses such as cytomegalovirus [78] and et. that consist of conjugation with the antibodies. The antibody mentioned in the paper of David G. et al. could mediate targeted killing even with the noncancer antigen peptides, via

reprogramming antigenicity of the tumor cells to increase effectiveness of immunotherapies [78]. In addition, there was also a report that links nanoparticles instead of antibodies to enhance immunogenicity [79].

Adjuvant modification

Peptide vaccines for inducing the CTL elimination were mainly designed similar with those conventional ones that were administrated by subcutaneous injection. As a reason, the adjuvants also play essential roles for the epitope-targeted vaccines such as the widely employed Freund's adjuvant (complete: CFA or incomplete: IFA) that mediate an inflammatory response [80-82]; the polyinosinic-polycytidylic acid (polyI:C) stabilized by lysine and carboxymethylcellulose (poly-ICLC) that promotes the infiltration of effector CTL [83, 84]; the biological protein adjuvant heat shock protein includes the recently defined HSP110 that reported a stronger protein binding affinity [85]; the factor adjuvants including the N-terminal end improving granulocyte-macrophage colony stimulating factor (GM-CSF) [86] and many other similar adjuvants that to assist the enhancement of the immune reactivity.

Another kind of adjuvants works by directly contacting and assisting the peptides to be more efficient: Liposomal cationic adjuvant formulation and nanodiscs are examples that conjugates the peptides by the liposomes to exert the strong CTL-inducing ability of the peptides, yet the former specifically requires intraperitoneal administration [87-89].

Peptide multimers

To employ the epitopes more efficiently, forming multimers of the peptides are also commonly used approaches. MHC-peptide tetramers were widely used for its reliable quantity assessment of the aimed immune cells [90, 91]. In fact, even only the peptide multimers can improve the efficiency of the combination via increasing the utilization ratio of the peptides. A trimeric long peptide conjugation was recently developed to enhance immunogenicity based on a trimers' platform, as its tertiary structure could increase the antigen presenting compared with the single peptide [92]. Such peptide multimer synthetic pattern was extended from the method called multiple antigen peptide (MAP) [93]. Though the initial purpose of the method was to multimerize different types of peptide antigens for better delivery, the application of MAPs in the research of Schetters STT et al. showed its immunotherapy potential on single peptide trimer [92].

Conclusion

Comparing among all the existing computational methods, most of the algorithms for predicting the epitope peptide cannot solve the problem of the deviation between the predicted results and the actual experimental outcomes. Although the IEDB database developers were focusing on increasing the data sets with bigger populations to improve its accuracy all these years, still only about half of the predictions are reasonable and reliable, which is another cause of the widely employing of the LC-MS methods. As a reason, to screen out the targeted tumor-antigen peptide with better accuracy, comprehensive use of multiple methods shall be preferred. In order to avoid gaining the inaccuracy results from the in silico tools because of lack of data, researchers shall turn to those tools that recently updated with steady updating records. In a similar way, suitable chromatography choosing can also improve the prediction accuracy. The former have reported that the comprehensive use of similar ligand omics experiments assays for the LC-MS/MS outcome analysis would improve both the FDR estimation and the number of identified peptides for MHC [94]. Despite the predictions, the modifications for the epitope peptides have been developing rapidly as well. A variety of methods are now available for enhancing the treatment effect, including different adjuvants and conjugation patterns. Yet special attention was necessary since the modification shall be based on the existing clinical results and suitable for the specified tumor.

With the development of the new TAA peptides [95], more and more tumor epitope peptide vaccines are now gradually putting into clinical trials. A few MHC-I anti-cancer peptides have entered the clinical trials after a series of in vitro screenings so far and the majority of them can successfully complete the clinical phase II trial, illustrating their safety and effectivity in clinical tumor treatment. Those peptides, that with great potential to pass clinical tests, would

make the peptide vaccine an efficient and safe therapy. As technology develops, the prediction methods of the epitope in tumor immunotherapy are likely to be more individual such as neoantigens and neoepitopes, that amounts of research are now focusing on them [96]. Yet these personalized treatmentsare usually with higher cost on price and time, which makes them hard to be popularized. By contrast, the broad-spectrum predictions mentioned we reviewed can give results more promptly without delaying the cancer treatment. But with the time going, the cost of personal therapy will ultimately be reduced in the future, then there will be a strong potential to have a new trend of the epitope and related applications. Based on that, epitope peptides derived from the predictions might be widely used as cancer treating therapy soon.

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Disclosure of conflict of interest

None.

Abbreviations

MHC, major histocompatibility complex; APC, antigen presenting cell; CTL, cytotoxic CD8+ T lymphocyte; TCR, T cell receptor; TA, tumor antigens; TAA, tumor-associated antigen; TSA, tumor-specific antigen; pMHC, peptide-MHC; ER, endoplasmic reticulum; TAP, transporter associated with antigen processing; TEIPP, T-cell epitopes associated with impaired peptide processing; SPP, signal peptide peptidase; ppCT, preprocalcitonin; HLA, human leukocyte antigen; MS, mass spectrometry; AE, acid elution; IP, immunoprecipitation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MS/MS, tandem mass spectrometry; FDR, false discovery rate; FACS, fluorescence-activated cell sorting; p3P, position 3 proline-altered; MAP, multiple antigen peptide.

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References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
[2] Mellman I, Coukos G and Dranoff G. Cancer immunotherapy comes of age. Nature 2011; 480: 480-489.
[3] Henderson RA, Mossman S, Nairn N and Cheever MA. Cancer vaccines and immunotherapies: emerging perspectives. Vaccine 2005; 23: 2359-2362.
[4] Aichele P, Hengartner H, Zinkernagel RM and Schulz M. Antiviral cytotoxic T cell response induced by in vivo priming with a free synthetic peptide. J Exp Med 1990; 171: 1815-1820.
[5] Thundimadathil J. Cancer treatment using peptides: current therapies and future prospects. J Amino Acids 2012; 2012: 967347.
[6] Blum JS, Wearsch PA and Cresswell P. Pathways of antigen processing. Annu Rev Immunol 2013; 31: 443-473.
[7] Shastri N, Schwab S and Serwold T. Producing nature's gene-chips: the generation of peptides for display by MHC class I molecules. Annu Rev Immunol 2002; 20: 463-493.
[8] Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, Thatcher N, Wagstaff J, Zielinski C, Faulkner I and Mellstedt H. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014; 11: 509-524.
[9] Marijt KA, Doorduijn EM and van Hall T. TEIPP antigens for T-cell based immunotherapy of immune-edited HLA class I(low) cancers. Mol Immunol 2019; 113: 43-49.
[10] Oliveira CC and van Hall T. Alternative antigen processing for MHC class I: multiple roads lead to rome. Front Immunol 2015; 6: 298.
[11] Leclerc M, Mezquita L, Guillebot De Nerville G, Tihy I, Malenica I, Chouaib S and Mami-Chouaib F. Recent advances in lung cancer immunotherapy: input of T-cell epitopes associ-

ated with impaired peptide processing. Front Immunol 2019; 10: 1505.
[12] Scott JK. Discovering peptide ligands using epitope libraries. Trends Biochem Sci 1992; 17: 241-245.
[13] Blake J, Johnston JV, Hellström KE, Marquardt H and Chen L. Use of combinatorial peptide libraries to construct functional mimics of tumor epitopes recognized by MHC class I-restricted cytolytic T lymphocytes. J Exp Med 1996; 184: 121-130.
[14] Leone P, Shin EC, Perosa F, Vacca A, Dammacco F and Racanelli V. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. J Natl Cancer Inst 2013; 105: 1172-1187.
[15] Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, Wheeler DK, Gabbard JL, Hix D, Sette A and Peters B. The immune epitope database (IEDB) 3.0. Nucleic Acids Res 2015; 43: D405-412.
[16] Liao WW and Arthur JW. Predicting peptide binding to major histocompatibility complex molecules. Autoimmun Rev 2011; 10: 469-473.
[17] Jones DT. Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol 1999; 292: 195-202.
[18] Andreatta M, Alvarez B and Nielsen M. GibbsCluster: unsupervised clustering and alignment of peptide sequences. Nucleic Acids Res 2017; 45: W458-w463.
[19] Rammensee H, Bachmann J, Emmerich NP, Bachor OA and Stevanović S. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 1999; 50: 213-219.
[20] Reche PA, Glutting JP and Reinherz EL. Prediction of MHC class I binding peptides using profile motifs. Hum Immunol 2002; 63: 701-709.
[21] Parker KC, Bednarek MA and Coligan JE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 1994; 152: 163-175.
[22] Tsukahara T, Kawaguchi S, Torigoe T, Takahashi A, Murase M, Kano M, Wada T, Kaya M, Nagoya S, Yamashita T and Sato N. HLA-A*0201-restricted CTL epitope of a novel osteosarcoma antigen, papillomavirus binding factor. J Transl Med 2009; 7: 44.
[23] Boehm KM, Bhinder B, Raja VJ, Dephoure N and Elemento O. Predicting peptide presentation by major histocompatibility complex class I: an improved machine learning approach to the immunopeptidome. BMC Bioinformatics 2019; 20: 7.
[24] Mei S, Li F, Leier A, Marquez-Lago TT, Giam K, Croft NP, Akutsu T, Smith AI, Li J, Rossjohn J, Purcell AW and Song J. A comprehensive review and performance evaluation of bioinformatics tools for HLA class I peptide-binding prediction. Brief Bioinform 2020; 21: 1119-1135.
[25] Ruppert J, Sidney J, Celis E, Kubo RT, Grey HM and Sette A. Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. Cell 1993; 74: 929-937.
[26] Zhang S, Chen J, Hong P, Li J, Tian Y, Wu Y and Wang S. PromPDD, a web-based tool for the prediction, deciphering and design of promiscuous peptides that bind to HLA class I molecules. J Immunol Methods 2020; 476: 112685.
[27] Bonsack M, Hoppe S, Winter J, Tichy D, Zeller C, Küpper MD, Schitter EC, Blatnik R and Riemer AB. Performance evaluation of MHC class-I binding prediction tools based on an experimentally validated MHC-peptide binding data set. Cancer Immunol Res 2019; 7: 719-736.
[28] Maclin PS, Dempsey J, Brooks J and Rand J. Using neural networks to diagnose cancer. J Med Syst 1991; 15: 11-19.
[29] Adams HP and Koziol JA. Prediction of binding to MHC class I molecules. J Immunol Methods 1995; 185: 181-190.
[30] Dönnes P and Elofsson A. Prediction of MHC class I binding peptides, using SVMHC. BMC Bioinformatics 2002; 3: 25.
[31] Wan J, Liu W, Xu Q, Ren Y, Flower DR and Li T. SVRMHC prediction server for MHC-binding peptides. BMC Bioinformatics 2006; 7: 463.
[32] Jurtz V, Paul S, Andreatta M, Marcatili P, Peters B and Nielsen M. NetMHCpan-4.0: improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. J Immunol 2017; 199: 3360-3368.
[33] Reynisson B, Alvarez B, Paul S, Peters B and Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res 2020; 48: W449-W454.
[34] Phloyphisut P, Pornputtapong N, Sriswasdi S and Chuangsuwanich E. MHCSeqNet: a deep neural network model for universal MHC binding prediction. BMC Bioinformatics 2019; 20: 270.
[35] Wang G, Wan H, Jian X, Li Y, Ouyang J, Tan X, Zhao Y, Lin Y and Xie L. INeo-Epp: a novel T-cell HLA class-I immunogenicity or neoantigenic epitope prediction method based on sequence-related amino acid features. Biomed Res Int 2020; 2020: 5798356.
[36] Sugawara S, Abo T and Kumagai K. A simple method to eliminate the antigenicity of surface class I MHC molecules from the membrane of

viable cells by acid treatment at pH 3. J Immunol Methods 1987; 100: 83-90.
[37] Storkus WJ, Zeh HJ 3rd, Salter RD and Lotze MT. Identification of T-cell epitopes: rapid isolation of class I-presented peptides from viable cells by mild acid elution. J Immunother Emphasis Tumor Immunol 1993; 14: 94-103.
[38] Feola S, Chiaro J, Martins B and Cerullo V. Uncovering the tumor antigen landscape: what to know about the discovery process. Cancers (Basel) 2020; 12: 1660.
[39] Kote S, Pirog A, Bedran G, Alfaro J and Dapic I. Mass spectrometry-based identification of MHC-associated peptides. Cancers (Basel) 2020; 12: 535.
[40] Hunt DF, Henderson RA, Shabanowitz J, Sakaguchi K, Michel H, Sevilir N, Cox AL, Appella E and Engelhard VH. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 1992; 255: 1261-1263.
[41] Bassani-Sternberg M, Pletscher-Frankild S, Jensen LJ and Mann M. Mass spectrometry of human leukocyte antigen class I peptidomes reveals strong effects of protein abundance and turnover on antigen presentation. Mol Cell Proteomics 2015; 14: 658-673.
[42] Schumacher FR, Delamarre L, Jhunjhunwala S, Modrusan Z, Phung QT, Elias JE and Lill JR. Building proteomic tool boxes to monitor MHC class I and class II peptides. Proteomics 2017; 17.
[43] Khodadoust MS, Olsson N, Wagar LE, Haabeth OA, Chen B, Swaminathan K, Rawson K, Liu CL, Steiner D, Lund P, Rao S, Zhang L, Marceau C, Stehr H, Newman AM, Czerwinski DK, Carlton VE, Moorhead M, Faham M, Kohrt HE, Carette J, Green MR, Davis MM, Levy R, Elias JE and Alizadeh AA. Antigen presentation profiling reveals recognition of lymphoma immunoglobulin neoantigens. Nature 2017; 543: 723-727.
[44] Faridi P, Purcell AW and Croft NP. In immunopeptidomics we need a sniper instead of a shotgun. Proteomics 2018; 18: e1700464.
[45] Purcell AW, Ramarathinam SH and Ternette N. Mass spectrometry-based identification of MHC-bound peptides for immunopeptidomics. Nat Protoc 2019; 14: 1687-1707.
[46] Eng JK, McCormack AL and Yates JR. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J Am Soc Mass Spectrom 1994; 5: 976-989.
[47] Cox J and Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol 2008; 26: 1367-1372.
[48] Song X, Bandow J, Sherman J, Baker JD, Brown PW, McDowell MT and Molloy MP. iTRAQ experimental design for plasma biomarker discovery. J Proteome Res 2008; 7: 2952-2958.
[49] Casado-Vela J, Martínez-Esteso MJ, Rodriguez E, Borrás E, Elortza F and Bru-Martínez R. iTRAQ-based quantitative analysis of protein mixtures with large fold change and dynamic range. Proteomics 2010; 10: 343-347.
[50] Ebrahimi-Nik H, Michaux J, Corwin WL, Keller GL, Shcheglova T, Pak H, Coukos G, Baker BM, Mandoiu II, Bassani-Sternberg M and Srivastava PK. Mass spectrometry driven exploration reveals nuances of neoepitope-driven tumor rejection. JCI Insight 2019; 5: e129152.
[51] Shao W, Pedrioli PGA, Wolski W, Scurtescu C, Schmid E, Vizcaíno JA, Courcelles M, Schuster H, Kowalewski D, Marino F, Arlehamn CSL, Vaughan K, Peters B, Sette A, Ottenhoff THM, Meijgaarden KE, Nieuwenhuizen N, Kaufmann SHE, Schlapbach R, Castle JC, Nesvizhskii AI, Nielsen M, Deutsch EW, Campbell DS, Moritz RL, Zubarev RA, Ytterberg AJ, Purcell AW, Marcilla M, Paradela A, Wang Q, Costello CE, Ternette N, van Veelen PA, van Els C, Heck AJR, de Souza GA, Sollid LM, Admon A, Stevanovic S, Rammensee HG, Thibault P, Perreault C, Bassani-Sternberg M, Aebersold R and Caron E. The SysteMHC atlas project. Nucleic Acids Res 2018; 46: D1237-D1247.
[52] Andreatta M, Nicastri A, Peng X, Hancock G, Dorrell L, Ternette N and Nielsen M. MS-rescue: a computational pipeline to increase the quality and yield of immunopeptidomics experiments. Proteomics 2019; 19: e1800357.
[53] Murphy JP, Konda P, Kowalewski DJ, Schuster H, Clements D, Kim Y, Cohen AM, Sharif T, Nielsen M, Stevanovic S, Lee PW and Gujar S. MHC-I ligand discovery using targeted database searches of mass spectrometry data: implications for T-cell immunotherapies. J Proteome Res 2017; 16: 1806-1816.
[54] Singh A. Genome-wide high-throughput screening of T cell epitopes. Nat Methods 2019; 16: 953.
[55] Sharma G, Rive CM and Holt RA. Rapid selection and identification of functional CD8(+) T cell epitopes from large peptide-coding libraries. Nat Commun 2019; 10: 4553.
[56] Bachinsky MM, Guillen DE, Patel SR, Singleton J, Chen C, Soltis DA and Tussey LG. Mapping and binding analysis of peptides derived from the tumor-associated antigen survivin for eight HLA alleles. Cancer Immun 2005; 5: 6.
[57] Shingler WH, Chikoti P, Kingsman SM and Harrop R. Identification and functional validation of MHC class I epitopes in the tumor-asso-

ciated antigen 5T4. Int Immunol 2008; 20: 1057-1066.
[58] Lopez M, Ghidouche A, Rochas C, Godelaine D, Carrasco J, Colau D, Hames G, Montero-Julian FA, Coulie PG and Olive D. Identification of a naturally processed HLA-A*02:01-restricted CTL epitope from the human tumor-associated antigen Nectin-4. Cancer Immunol Immunother 2016; 65: 1177-1188.
[59] Baba T, Sato-Matsushita M, Kanamoto A, Itoh A, Oyaizu N, Inoue Y, Kawakami Y and Tahara H. Phase I clinical trial of the vaccination for the patients with metastatic melanoma using gp100-derived epitope peptide restricted to HLA-A*2402. J Transl Med 2010; 8: 84.
[60] Wulf M, Hoehn P and Trinder P. Identification and validation of T-cell epitopes using the IFN-gamma ELISPOT assay. Methods Mol Biol 2009; 524: 439-446.
[61] Bjorkman PJ and Parham P. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu Rev Biochem 1990; 59: 253-288.
[62] Anthony DD and Lehmann PV. T-cell epitope mapping using the ELISPOT approach. Methods 2003; 29: 260-269.
[63] Kosor E, Gagro A, Drazenović V, Kuzman I, Jeren T, Rakusić S, Rabatić S, Markotić A, Gotovac K, Sabioncello A, Cecuk E, Kerhin-Brkljacić V, Gjenero-Margan I, Kaić B, Mlinarić-Galinović G, Kastelan A and Dekaris D. MHC tetramers: tracking specific immunity. Acta Med Croatica 2003; 57: 255-259.
[64] Scheikl-Gatard T, Tosch C, Lemonnier F and Rooke R. Identification of new MUC1 epitopes using HLA-transgenic animals: implication for immunomonitoring. J Transl Med 2017; 15: 154.
[65] Sun W, Shi J, Wu J, Zhang J, Chen H, Li Y, Liu S, Wu Y, Tian Z, Cao X and Li N. A modified HLA-A*0201-restricted CTL epitope from human oncoprotein (hPEBP4) induces more efficient antitumor responses. Cell Mol Immunol 2018; 15: 768-781.
[66] Quayle SN, Girgis N, Thapa DR, Merazga Z, Kemp MM, Histed A, Zhao F, Moreta M, Ruthardt P, Hulot S, Nelson A, Kraemer LD, Beal DR, Witt L, Ryabin J, Soriano J, Haydock M, Spaulding E, Ross JF, Kiener PA, Almo S, Chaparro R, Seidel R, Suri A, Cemerski S, Pienta KJ and Simcox ME. CUE-101, a novel E7-pHLA-IL2-Fc fusion protein, enhances tumor antigen-specific T-cell activation for the treatment of HPV16-driven malignancies. Clin Cancer Res 2020; 26: 1953-1964.
[67] Li R, Zheng C, Wang Q, Bi E, Yang M, Hou J, Fu W, Yi Q and Qian J. Identification of an immunogenic DKK1 long peptide for immunotherapy of human multiple myeloma. Haematologica 2021; 106: 838-846.
[68] Ostankovitch M, Altrich-Vanlith M, Robila V and Engelhard VH. N-glycosylation enhances presentation of a MHC class I-restricted epitope from tyrosinase. J Immunol 2009; 182: 4830-4835.
[69] Madura F, Rizkallah PJ, Legut M, Holland CJ, Fuller A, Bulek A, Schauenburg AJ, Trimby A, Hopkins JR, Wells SA, Godkin A, Miles JJ, Sami M, Li Y, Liddy N, Jakobsen BK, Loveridge EJ, Cole DK and Sewell AK. TCR-induced alteration of primary MHC peptide anchor residue. Eur J Immunol 2019; 49: 1052-1066.
[70] Bristol JA, Schlom J and Abrams SI. Development of a murine mutant Ras CD8+ CTL peptide epitope variant that possesses enhanced MHC class I binding and immunogenic properties. J Immunol 1998; 160: 2433-2441.
[71] Tang Y, Lin Z, Ni B, Wei J, Han J, Wang H and Wu Y. An altered peptide ligand for naïve cytotoxic T lymphocyte epitope of TRP-2(180-188) enhanced immunogenicity. Cancer Immunol Immunother 2007; 56: 319-329.
[72] Sachs A, Moore E, Kosaloglu-Yalcin Z, Peters B, Sidney J, Rosenberg SA, Robbins PF and Sette A. Impact of cysteine residues on MHC binding predictions and recognition by tumor-reactive T cells. J Immunol 2020; 205: 539-549.
[73] Duru AD, Sun R, Allerbring EB, Chadderton J, Kadri N, Han X, Peqini K, Uchtenhagen H, Madhurantakam C, Pellegrino S, Sandalova T, Nygren P, Turner SJ and Achour A. Tuning antiviral CD8 T-cell response via proline-altered peptide ligand vaccination. PLoS Pathog 2020; 16: e1008244.
[74] Vhora I, Patil S, Bhatt P and Misra A. Protein- and Peptide-drug conjugates: an emerging drug delivery technology. Adv Protein Chem Struct Biol 2015; 98: 1-55.
[75] Wang Y, Cheetham AG, Angacian G, Su H, Xie L and Cui H. Peptide-drug conjugates as effective prodrug strategies for targeted delivery. Adv Drug Deliv Rev 2017; 110-111: 112-126.
[76] King BC, Hamblin AD, Savage PM, Douglas LR, Hansen TH, French RR, Johnson PW and Glennie MJ. Antibody-peptide-MHC fusion conjugates target non-cognate T cells to kill tumour cells. Cancer Immunol Immunother 2013; 62: 1093-1105.
[77] Masterman KA, Haigh OL, Tullett KM, Leal-Rojas IM, Walpole C, Pearson FE, Cebon J, Schmidt C, O'Brien L, Rosendahl N, Daraj G, Caminschi I, Gschweng EH, Hollis RP, Kohn DB, Lahoud MH and Radford KJ. Human CLEC9A antibodies deliver NY-ESO-1 antigen to CD141(+) dendritic cells to activate naïve and memory NY-ESO-1-specific CD8(+) T cells. J Immunother Cancer 2020; 8: e000691.
[78] Millar DG, Ramjiawan RR, Kawaguchi K, Gupta N, Chen J, Zhang S, Nojiri T, Ho WW, Aoki S, Jung K, Chen I, Shi F, Heather JM, Shigeta K,

Morton LT, Sepulveda S, Wan L, Joseph R, Minogue E, Khatri A, Bardia A, Ellisen LW, Corcoran RB, Hata AN, Pai SI, Jain RK, Fukumura D, Duda DG and Cobbold M. Antibody-mediated delivery of viral epitopes to tumors harnesses CMV-specific T cells for cancer therapy. Nat Biotechnol 2020; 38: 420-425.

[79] Lang S, Tan Z, Wu X and Huang X. Synthesis of carboxy-dimethylmaleic amide linked polymer conjugate based ultra-pH-sensitive nanoparticles for enhanced antitumor immunotherapy. ACS Macro Lett 2020; 9: 1693-1699.

[80] Reitermann A, Metzger J, Wiesmüller KH, Jung G and Bessler WG. Lipopeptide derivatives of bacterial lipoprotein constitute potent immune adjuvants combined with or covalently coupled to antigen or hapten. Biol Chem Hoppe Seyler 1989; 370: 343-352.

[81] Bessler WG and Jung G. Synthetic lipopeptides as novel adjuvants. Res Immunol 1992; 143: 548-553; discussion 579-580.

[82] Wang E, Phan GQ and Marincola FM. T-cell-directed cancer vaccines: the melanoma model. Expert Opin Biol Ther 2001; 1: 277-290.

[83] Zhu X, Fallert-Junecko BA, Fujita M, Ueda R, Kohanbash G, Kastenhuber ER, McDonald HA, Liu Y, Kalinski P, Reinhart TA, Salazar AM and Okada H. Poly-ICLC promotes the infiltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN-alpha and IFN-gamma dependent manners. Cancer Immunol Immunother 2010; 59: 1401-1409.

[84] Sultan H, Kumai T, Nagato T, Wu J, Salazar AM and Celis E. The route of administration dictates the immunogenicity of peptide-based cancer vaccines in mice. Cancer Immunol Immunother 2019; 68: 455-466.

[85] Ding Z, Ou R, Ni B, Tang J and Xu Y. Cytolytic activity of the human papillomavirus type 16 E711-20 epitope-specific cytotoxic T lymphocyte is enhanced by heat shock protein 110 in HLA-A*0201 transgenic mice. Clin Vaccine Immunol 2013; 20: 1027-1033.

[86] Butterfield LH, Zhao F, Lee S, Tarhini AA, Margolin KA, White RL, Atkins MB, Cohen GI, Whiteside TL, Kirkwood JM and Lawson DH. Immune correlates of GM-CSF and melanoma peptide vaccination in a randomized trial for the adjuvant therapy of resected high-risk melanoma (E4697). Clin Cancer Res 2017; 23: 5034-5043.

[87] Korsholm KS, Hansen J, Karlsen K, Filskov J, Mikkelsen M, Lindenstrøm T, Schmidt ST, Andersen P and Christensen D. Induction of CD8+ T-cell responses against subunit antigens by the novel cationic liposomal CAF09 adjuvant. Vaccine 2014; 32: 3927-3935.

[88] Schmidt ST, Olsen CL, Franzyk H, Wørzner K, Korsholm KS, Rades T, Andersen P, Foged C and Christensen D. Comparison of two different PEGylation strategies for the liposomal adjuvant CAF09: towards induction of CTL responses upon subcutaneous vaccine administration. Eur J Pharm Biopharm 2019; 140: 29-39.

[89] Kuai R, Ochyl LJ, Bahjat KS, Schwendeman A and Moon JJ. Designer vaccine nanodiscs for personalized cancer immunotherapy. Nat Mater 2017; 16: 489-496.

[90] Leisner C, Loeth N, Lamberth K, Justesen S, Sylvester-Hvid C, Schmidt EG, Claesson M, Buus S and Stryhn A. One-pot, mix-and-read peptide-MHC tetramers. PLoS One 2008; 3: e1678.

[91] Zhu J, Li Y, Li L, Wang J, Wang H, Hong W, Hao K, Xue Y, Chen B and Wang Z. A novel absorption spectrometric method, based on graphene nanomaterials, for detection of hepatocellular carcinoma-specific T lymphocyte cells. Int J Nanomedicine 2018; 13: 5523-5536.

[92] Schetters STT, Li RJE, Kruijssen LJW, Engels S, Ambrosini M, Garcia-Vallejo JJ, Kalay H, Unger WWJ and van Kooyk Y. Adaptable antigen matrix platforms for peptide vaccination strategies and T cell-mediated anti-tumor immunity. Biomaterials 2020; 262: 120342.

[93] Tam JP. Recent advances in multiple antigen peptides. J Immunol Methods 1996; 196: 17-32.

[94] Konda P, Murphy JP and Gujar S. Improving MHC-I ligand identification by incorporating targeted searches of mass spectrometry data. Methods Mol Biol 2020; 2120: 161-171.

[95] Berlin C, Kowalewski DJ, Schuster H, Mirza N, Walz S, Handel M, Schmid-Horch B, Salih HR, Kanz L, Rammensee HG, Stevanović S and Stickel JS. Mapping the HLA ligandome landscape of acute myeloid leukemia: a targeted approach toward peptide-based immunotherapy. Leukemia 2015; 29: 647-659.

[96] Fritsch EF, Burkhardt UE, Hacohen N and Wu CJ. Personal neoantigen cancer vaccines: a road not fully paved. Cancer Immunol Res 2020; 8: 1465-1469.

[97] Yu H, Ye C, Li J, Pan C, Lin W, Chen H, Zhou Z and Ye Y. An altered HLA-A0201-restricted MUC1 epitope that could induce more efficient anti-tumor effects against gastric cancer. Exp Cell Res 2020; 390: 111953.

[98] Nezafat N, Ghasemi Y, Javadi G, Khoshnoud MJ and Omidinia E. A novel multi-epitope peptide vaccine against cancer: an in silico approach. J Theor Biol 2014; 349: 121-134.

[99] Krishna S, Ulrich P, Wilson E, Parikh F, Narang P, Yang S, Read AK, Kim-Schulze S, Park JG, Posner M, Wilson Sayres MA, Sikora A and Anderson KS. Human papilloma virus specific immunogenicity and dysfunction of CD8(+) T

cells in head and neck cancer. Cancer Res 2018; 78: 6159-6170.
[100] Jaeger AM, Stopfer L, Lee S, Gaglia G, Sandel D, Santagata S, Lin NU, Trepel JB, White F, Jacks T, Lindquist S and Whitesell L. Rebalancing protein homeostasis enhances tumor antigen presentation. Clin Cancer Res 2019; 25: 6392-6405.
[101] Jabbar B, Rafique S, Salo-Ahen OMH, Ali A, Munir M, Idrees M, Mirza MU, Vanmeert M, Shah SZ, Jabbar I and Rana MA. Antigenic peptide prediction from E6 and E7 oncoproteins of HPV types 16 and 18 for therapeutic vaccine design using immunoinformatics and MD simulation analysis. Front Immunol 2018; 9: 3000.
[102] Shraibman B, Kadosh DM, Barnea E and Admon A. Human leukocyte antigen (HLA) peptides derived from tumor antigens induced by inhibition of DNA methylation for development of drug-facilitated immunotherapy. Mol Cell Proteomics 2016; 15: 3058-3070.
[103] Tarek MM, Shafei AE, Ali MA and Mansour MM. Computational prediction of vaccine potential epitopes and 3-dimensional structure of XAGE-1b for non-small cell lung cancer immunotherapy. Biomed J 2018; 41: 118-128.
[104] Singh H and Raghava GP. ProPred1: prediction of promiscuous MHC Class-I binding sites. Bioinformatics 2003; 19: 1009-1014.
[105] Namvar A, Bolhassani A, Javadi G and Noormohammadi Z. In silico/In vivo analysis of high-risk papillomavirus L1 and L2 conserved sequences for development of cross-subtype prophylactic vaccine. Sci Rep 2019; 9: 15225.