



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Review

Peptide-based immunotherapy for multiple myeloma: Current approaches

Fu-ling Zhou^{a,*}, Shan Meng^a, Wang-gang Zhang^a, Yong-chang Wei^b, Xing-mei Cao^a,
Gai-gai Bai^a, Bai-yan Wang^c

^a The Department of Clinical Hematology, The Affiliated No. 2 Hospital, Xi'an Jiaotong University, The West Five Road, No. 157, Xi'an 710004, PR China

^b The Department of Clinical Oncology, The Affiliated No. 1 Hospital, Xi'an Jiaotong University, The Jian-Kang Road, No. 1, Xi'an 710061, PR China

^c Shanghai Institute of Hematology, Rui-jin Hospital, School Medicine, Shanghai Jiaotong University, 197 Rui Jin Er Road, Shanghai 200025, PR China

ARTICLE INFO

Article history:

Received 26 March 2010
Received in revised form 12 June 2010
Accepted 28 June 2010
Available online xxx

Keywords:

Peptide
Epitope
Multiple Myeloma
Immunotherapy

ABSTRACT

Multiple myeloma (MM) is a clonal B-cell malignancy with many fatal clinical sequelae. Despite extensive therapeutic approaches, cures remain rare exceptions. A recent promising area of investigation is the development of immunotherapeutic approaches that target and eliminate myeloma cells more selectively. Because of its potential to promote the destruction of cancerous cells via cytotoxic T-cell responses, peptide-based immunotherapy is one of these strategies to have attracted considerable attention. Furthermore, many studies were carried out to identify the best epitope peptides, the optimal vaccine formulation and schedule, and the preferable clinical situation for vaccination. Based on these results, various epitope peptides have been identified that may be selectively targeted by host immunity, and various approaches have been used to enhance the immune responses of peptides. This chapter focuses on reviewing previous immunotherapy trials, describing the current strategies for peptide-based immunotherapy, and discussing the achievable prospects in MM.

© 2010 Elsevier Ltd. All rights reserved.

Contents

1. Introduction.....	00
2. Application of peptide-based immunotherapy in MM trials.....	00
2.1. Tumor-associated antigens.....	00
2.2. Cancer-testis antigen.....	00
2.3. Tumor-specific antigen.....	00
2.4. Transcription factors.....	00
2.5. Immunomodulating peptide.....	00
3. Advanced approaches for enhancing the immune response.....	00
3.1. Adjuvants.....	00
3.2. Bioinformatics tools.....	00
3.3. Modified epitope peptide.....	00
3.4. Microspheres.....	00
3.5. Adoptive therapy for tumor immunity.....	00
3.6. Recombinant bacteriophages.....	00
3.7. Cell-penetrating peptides.....	00
3.8. Multiple antigen peptides.....	00
4. Concluding remarks.....	00
Conflict of interest statement.....	00
Acknowledgment.....	00
References.....	00

* Corresponding author. Tel.: +86 29 87679457; fax: +86 29 87678634.
E-mail address: zhoufuling@163.com (F.-l. Zhou).

1. Introduction

Multiple myeloma (MM) is characterized by clonal proliferation of plasma cells in the bone marrow (BM) usually associated with elevated serum and urine monoclonal paraprotein. Studies over the past few years have demonstrated that increasing knowledge of the underlying biological mechanisms has led the way for the development of novel chemotherapy drugs and transplantation methods, and options for the management of MM are rapidly evolving [1,2]. However, MM continues to represent an incurable disease with fatal outcomes in the majority of patients in advanced stages, highlighting the need for novel therapeutic agents.

MM is an immunologically relevant disease that subverts and suppresses immunity. Despite its highly complex nature, the exploration of novel therapeutic modalities has been pursued in the field of tumor immunotherapy. Notably, many clinical and animal trials have evaluated the therapeutic value of immunotherapy, and the safety of peptide-based immunotherapy targeting B cells and plasma cells has been demonstrated. Peptide vaccines, in which small epitope peptides derived from target proteins are used to provoke an immune reaction, have attracted considerable attention as a potential means of promoting the destruction of cancerous cells [3]. Moreover, combinatorial approaches in which vaccines are used to decrease the tumor burden and modulate regulatory pathways may eventually be clinically useful. This article summarizes the important components of peptide-based immunotherapy for MM and reviews advanced approaches that are being attempted, with an emphasis on the effects of polypeptide vaccines.

2. Application of peptide-based immunotherapy in MM trials

There is growing evidence that peptide-based immune interventions are promising for MM, with many easily produced peptides now being developed (Table 1). The data suggest the immunogenicity of the target antigen and that immunotherapeutic strategies using peptides might be an option for patients with MM. The use of synthetic peptides as vaccines is aimed at the induction of therapeutic responses against tumor cells. Meanwhile, synthetic peptides are chemically stable and free of bacterial/viral contaminating substances and are devoid of oncogenic potential. Importantly, the simplicity of producing clinical-grade peptides allows rapid changes to the design of peptide vaccines and, therefore, rapid translation of new immunological concepts into phase I/II trials in humans. In addition, the use of synthetic peptides offers an alternative to proteins, which are currently difficult to produce by recombinant technology due to their inherent toxicity toward the bacterial/viral expression system.

2.1. Tumor-associated antigens

In general, MM has been considered a weakly immunogenic malignancy that can cause profound defects in the immune system. Recent advances in tumor antigen discovery and epitope design are making it possible to streamline the production of effective immunotherapy. In particular, various tumor-associated antigens (TAA) that have been identified in myelomas that may be selectively targetable by the host immune system, and the results of preclinical and clinical trials have suggested their validity and advantages.

Wilms' tumor gene (WT1) possesses oncogenic functions and is expressed in various kinds of malignancies. To date, many WT1

peptides have previously been shown to elicit a WT1-specific CTL response with a restriction of HLA-A*2402, HLA-A*0201, HLA-A*0206 and so on [4–6]. As a promising target antigen for cancer immunotherapy, the WT1 peptide achieved a vaccination-driven response including reduction of MM cells, reduction of the M-protein amount in myeloma, improvement of bone, and fewer adverse effects [6]. Moreover, the WT1 peptide vaccine in an “adjuvant setting” was considered an encouraging treatment to protect against the progression or relapse of malignancies in cases with minimal residual disease, and long-term stabilization of disease with good quality of life was also observed [7].

Mucin1 (MUC1) is an epithelial cell antigen whose aberrant expression plays roles in the autoimmunity and tumor immunity of MM [8]. Brossart et al. [9] identified two HLA-A2-restricted T-cell epitopes derived from MUC1 protein, and MUC1-specific CTLs efficiently lysed blast cells from MM patients *in vitro* in an antigen-specific and HLA-A2-restricted manner. Choi et al. [10] analyzed the PB or BM of 68 patients with HLA-A2-positive MM for the presence and functional activity of CD8 T cells specific for the MUC1-derived peptide. The results exhibited enrichment of functional CD8 memory T cells specific for MUC1 in the bone marrow of patients with MM. In addition, the functional capacity upon specific reactivation with regard to interferon γ (IFN- γ) secretion, perforin production, and cytotoxicity was higher for BM-residing T cells than for PB-derived T cells.

Human telomerase reverse transcriptase (hTERT) is detected in most human malignancies, and a strong correlation between telomerase activation and hTERT expression is considered to be a critical step in carcinogenesis. Kryukov et al. [11] found that an HLA-A2-specific hTERT-derived nonapeptide could act as a TAA, and established that hTERT-reactive CTLs exhibited cytotoxic activity against ARH77, an established human IgG-producing myeloma cell line, *in vitro*. Furthermore, T cells obtained from HLA-A2 blood donors were repeatedly stimulated with hTERT and MUC1 nonapeptide-loaded dendritic cells. Activated myeloma-specific T cells can produce IFN- γ , and their efficacy in MM therapy has been demonstrated [12].

The receptor for hyaluronic-acid-mediated motility (RHAMM) is an antigen that elicits both humoral and cellular immune responses in patients with hematologic disorder, including MM. Schmitt et al. [13] initiated a phase I clinical trial involving the vaccination of 10 patients with a nonapeptide (a highly immunogenic CD8(+) T-cell epitope peptide derived from RHAMM-R3) and found that the serum levels of free light chain was reduced in 2 of 4 patients with MM.

Dickkopf-1 (DKK1) is a soluble inhibitor of Wnt that disrupts osteoblast differentiation and action. DKK1 plays pivotal roles in bone health and disease and is a novel target for managing MM patients with lytic bone disease [14]. For example, Qian et al. [15] identified and synthesized DKK1 peptides for HLA-A*0201 and confirmed their immunogenicity by *in vivo* immunization in HLA-A*0201 transgenic mice. They detected low frequencies of DKK1 peptide-specific CD8(+) T cells in patients with MM and generated peptide-specific T-cell lines. These T cells were potent cytotoxic T cells that efficiently lysed peptide-pulsed dendritic cells, DKK1(+)/HLA-A*0201(+) myeloma cell lines U266 and IM-9, and, more importantly, HLA-A*0201(+) primary myeloma cells from patients.

Survivin is a member of the family of apoptosis inhibitors. Grube et al. [16] found positive survivin expression in myeloma cells from BM specimens in 7 of 11 patients. T cells recognizing HLA-A2.1-binding survivin peptide were detected in 9 of 23 patients with MM and in 1 of 21 healthy volunteers. Moreover, survivin-reactive T cells producing IFN- γ were identified as terminally differentiated effector T cells [CD8(+), CD45RA(+), and CCR7(–)].

Table 1

HLA restrictions of peptide sequences used in immunotherapies against MM.

Origin	Amino acid position	Peptide sequences	HLA restriction	Trial type	Researchers
WT1	235–243	CMTWNQMNL	HLA-A*2402	Patients	[4]
	235–243	CMTWNQMNL	HLA-A*2402		[6]
	126–134	RMFPNAPYL	HLA-A*0201		[6]
	187–195	SLGEQQYSV	HLA-A*0201		[6]
	235–243	CMTWNQMNL	HLA-A*0201		[6]
MUC1	187–195	SLGEQQYSV	HLA-A*0206	Cell lines	[5]
	950–958	STAPPVHNV	HLA-A2		[9]
	12–20	LLLLTVLTV	HLA-A2		[9,10]
	540–548	ILAKFLHWL	HLA-A2		[11]
	165–173	ILSLELMKL	HLA-A*0201		[13]
RHAMM-R3	20–29	ALGGHPLLGV	HLA-A*0201	Cell lines	[15]
DKK1	20–29*y 20	YLGGHPLLGV			
	66–74	ILYPGGNKY			
	66–74*66v	ILYPGGNKV			
	58–66	GILGFVFTL			
	476–474	ILKEPVHGV			
Survivin	95–104	ELTLGEFLKL	HLA-A*0201	patients	[16]
PAK2	–	KLAKPLSSL	HLA-A2	Healthy donors	[19]
	–	VLLGMEGSV			
CDKN1A	–	GLGLPKLYL	HLA-A2	Healthy donors	[19]
	–	LMAGCIQEA			
	–	FAWERVERGL			
HM1.24	22–30	LLLGIGILV	HLA-A2	Cell lines	[17]
MAGE-A1	96–104	SLFRAVITK	HLA-A3	Patients	[24]
	135–143	NYKHCFPEI	HLA-A24		
	161–169	EADPTGHSY	HLA-A1		
	289–298	RVRFFFPSL	HLA*B0702		
	157–166	YLQLVFGIEV	HLA-A*0201		
MAGE-A2	97–105	TFPDLESEF	HLA-A24	Patients	[24,25]
MAGE-A3	112–120	KVAELVHFL	HLA-A2	Patients	[24]
	168–176	EVDPIGHLY	HLA-A1		
	195–203	IMPKAGLLI	HLA-A*2401		
	115–123	ELVHFLLK	HLA-A*6801		
	230–239	GVYDGREHTV	HLA-A2		
MAGE-A4	Alt-ORF	MLMAQEALAF	HLA-A*0201	Patients	[24]
LAGE-1	157–165	SLLMWITQC	HLA-A2	Patients	[24]
NY-ESO-1	157–165	SLLMWITQC	HLA-A*0201	Patients	[26]
	51–62	ARASGPGGGAPR	HLA-DQ5		
	121–140	VLLKEFTVSG	HLA-DR4		
	26–35	EAAGIGILTV	HLA-A2		
	26–35*A27L	ELAGIGILTV	HLA-A2		
SPAN-XB	624–712	KTSESSTIL	HLA-A2	Patients	[27]
	626–714	SESSTILVV	HLA-A2		
	626–714* E627 L	SLSSTILVV	HLA-A2		
	–	TLVTVSSAS	HLA-A2		
	–	LMISRTPEV	HLA-A2		
IgGH	–	FSLRTREMCVTWIRQ	HLA-DRB*10401	Patients	[31]
	–	SLKTRLTIS	HLA-B8		
	–	RIDWDDEKY	HLA-A1		
	–	GYPVTNAVI	HLA-A24		
	–	VFQHGKVEI			
Hsp70	136–144	FLLPPYGMNC	HLA-A*0201	Cell lines	[32]
PRDI-BF1	20–28	FGLFPRLCPV		Transgenic mice/cell lines	[34]
	377–386	GLFPRLCPV			
	401–410	LLSGQPASA			
XBP-1	402–410	LLSGQPASA		Transgenic mice/cell lines	[34]
	18–27	LLSGQPASA	HLA-A*0201		
	19–27	LLSGQPASA			

HM1.24 antigen was originally identified as a cell-surface protein that is preferentially overexpressed in MM cells. HM1.24 [amino acids (aa) 22–30] is a new HLA-A2-restricted T-cell epitope that is processed and presented by major histocompatibility complex class-I. Hundemer et al. [17] stimulated PB mononuclear cells (PBMCs) from HLA-A2(+) healthy volunteer blood donors with autologous HM1.24-peptide-loaded dendritic cells, and the HM1.24 [aa 22–30] peptide showed the most frequent activation in CD8(+) T cells in healthy volunteers. Importantly, HM1.24-specific CD8(+) T cells lysed HLA-A2(+) MM-derived cell lines.

The Melan-A (aa 26–35) peptide is an HLA-A2-restricted T-cell epitope within the Melan-A tumor antigen. Melan-A and Melan-A analog [ELAGIGILTV, Melan-A (aa 26–35*A27L)]-specific T cells can be expanded reliably for use in immunotherapeutic approaches *in*

vitro [18]. Christensen et al. also reported that Melan-A-analog-specific T cells could secrete IFN- γ mediated by human myeloma (HM)1.24 (aa 22–30) peptide-pulsed T2 cells and can lyse HLA-A2(+)HM1.24(+) U266 and XG-1-HM-derived cell lines, as well as the B-lymphoblastoid cell line IM-9. Melan-A-analog-specific T cells from MM patients specifically lysed autologous MM cells.

P21-activated serine kinase 2 (PAK2) and cyclin-dependent kinase inhibitor 1A (CDKN1A) are putative tumor-associated antigens of MM. PAK2- and CDKN1A-derived peptides can elicit a strong and consistent CD8(+) T-cell response in *in vitro* models. Li et al. [19] identified and characterized immunogenic peptides with strong binding to HLA-A2 in the amino acid sequences of PAK2 and CDKN1A. In an IFN- γ ELISPOT assay, all tested peptides demonstrated the presence and functional activity of CD8-peptide-specific T cells.

2.2. Cancer-testis antigen

Cancer-testis (CT) antigens are expressed in MM but not in healthy donors. The tumor-associated expression pattern of these antigens potentially makes them suitable for diagnostic applications and motivates major interest as potential targets for immunotherapy [20–25]. Andrade et al. [21] analyzed the expression of 14 CT antigens in 39 MM samples (95% at the advanced stage) and found frequencies of 77% for MAGE-C1/CT7. Furthermore, Atanackovic et al. [22] also concluded that MAGE-C1/CT7 might characterize a more malignant phenotype, representing an important indicator of early relapse and dramatically reduced survival. This conclusion is partly confirmed by Tinguely et al. [23], who reported that the expression of MAGE-C1/CT7 in PMs is positively correlated with proliferation rate and overall survival. Goodyear et al. [24] used 12 peptide epitopes from a range of CT antigens to screen for specific T cells in blood samples from patients with MM at various disease stages. T cells from 15 of 37 patients responded to one or more CT-antigen peptides, with the magnitude of the specific CD8(+) T-cell response ranging between 0.0004% and 0.1% of the total CD8(+) T-cell pool. In patients undergoing treatment or in disease relapse, the magnitude of the CT-antigen-specific T-cell response was positively correlated with the level of paraprotein. In addition, they found that CD8(+) T-cell immunity to MAGE proteins was present almost exclusively in patients with MM, and was able to lyse myeloma cell lines *in vitro* [25].

NY-ESO-1 is a CT antigen of particular interest because both CTLs and antibodies have been shown to react with this antigen. Spontaneous NY-ESO-1-specific T cells, which were found in the PB of NY-ESO-1(+) MM with HLA-A*0201 NY-ESO-1 (aa 157–165) tetramers, can kill primary MM cells when expanded [26]. Strong antibody responses against NY-ESO-1 correlated with NY-ESO-1-specific CD4(+) and CD8(+) T-cell responses against peptide NY-ESO-1 (aa 51–62), and CD4(+) responses against NY-ESO-1 (aa 121–140) were found in one patient who had received allogeneic stem-cell transplantation. Furthermore, immune responses induced by alloSCT could be boosted by active CT-antigen-specific immunotherapy, which might help to achieve long-lasting remission in patients with MM [20].

SPAN-XB is a novel CT antigen that exhibits distinctive immunogenicity. Frank et al. [27] identified two SPAN-XB-derived peptides (peptides 624 and 626) with predicted binding to HLA-A2, and they designed a heteroclitic peptide (peptide 627) by modifying one amino acid on the HLA-binding position 2 of peptide 626 to further improve the immunogenicity of this peptide. Using an IFN- γ ELISPOT assay, they demonstrated the presence and functional activity of CD8-peptide-specific T cells with all tested peptides. At least 1 of the tested peptides could be specifically detected and expanded in PB from 9 of the 13 tested healthy donors and 3 of the 5 tested MM patients with MM-peptide-specific T-cell precursors. Importantly, specific peptides could be generated against the heteroclitic peptide 627, but not against the native peptide 626 in two donors.

2.3. Tumor-specific antigen

Myeloma cells produce a monoclonal immunoglobulin that is a truly tumor-specific antigen. The tumor-specific antigenic determinants are localized in the variable regions of the monoclonal immunoglobulin and are termed idiotopes (Id). Preclinical studies have demonstrated that MM cells display idiotypic peptides on their surface in a form suitable for recognition and killing by host T cells, supporting the application of Id-vaccination in MM [28]. Cohen et al. [29] found that vaccination with Id-

pulsed dendritic cells (DCs) or with soluble Id-keyhole limpet hemocyanin(KLH) in adjuvant induced immune responses that eliminated myeloma in tumor-bearing mice. Hansson et al. [30] demonstrated that a large number of HLA-binding idiotype peptides could spontaneously induce both type I major histocompatibility complex (MHC) class-I- and class-II-restricted memory T-cell responses. Most of the predicted peptides were derived from the complementarity-determining region (CDR; 52–60%) rather than from the framework region (FR; 40–48%). Most of the predicted peptides were confined to the CDR2-FR3-CDR3 “geographic” region of the variable region of the immunoglobulin heavy chain (70%), with significantly fewer peptides being found within the flanking FR1-CDR1-FR2 and FR4 regions. Belle et al. [31] reported that two HLA-A2-restricted T-cell epitopes derived from IgGH-FR4 and the constant region induced expansion of specific CD8(+) T cells from PBMCs in healthy donors and patients with MM, respectively. Moreover, T-cell epitope-specific CD8(+) T cells from MM patients specifically lysed the HLA-A2(+) IgG myeloma cell line XG-6.

Members of the heat shock protein 70 (Hsp70) family are known to facilitate immune responses by interacting with receptors on antigen-presenting cells (APCs), leading to Hsp70-peptide uptake and antigen cross-priming. Hsp70 plays several roles in the adaptive immune response. Based on the ability to interact with diverse peptides, extracellular Hsp70:peptide complexes exert profound effects both in autoimmunity and in tumor rejection by evoking potent T cell responses to the chaperoned peptide. Okochi et al. [32] recently identified two HLA-A24-restricted epitopes using Hsp70 as the model antigen. These peptides actually bound to HLA-A24 in a stabilization assay using T2-A*2402 cells and induced a strong peptide-reactive CTL response in HLA-A24 transgenic mice after vaccination. Because HSP-associated peptides are known to mediate tumor rejection, immune-mediated eradication of MM cells could be elicited via an ultraviolet (UV)-induced HSP process. The novel method of priming CTLs with UV-irradiated tumor cells terminates tumor cell proliferation and apoptosis and upregulates HSP expression. PBMCs primed using UV-irradiated tumor cells demonstrated a higher percentage of activated CD8(+)/CD4(–) T cells and was markedly more effective at killing RPMI 8226 MM cells. These observations provide a framework for developing anti-tumor CTL vaccines for treating MM using UV-irradiated tumor cells as immunogens [33].

2.4. Transcription factors

Lotz et al. [34] concluded that transcription factors governing terminal cellular differentiation may provide MM-associated CTL epitopes. HLA-A*0201 transgenic mice were used to identify HLA-A*0201-presenting peptide antigen-derived from the plasma-cell-associated transcriptional regulators, positive regulatory domain-I-binding factor 1 (PRDI-BF1), and X-box-binding protein 1 (XBP-1). HLA-A*0201-restricted CTL specific for PRDI-BF1 and XBP-1 epitopes efficiently killed a variety of MM targets. PRDI-BF1- and XBP-1-reactive CTLs were able to recognize primary MM cells from HLA-A*0201 patients.

2.5. Immunomodulating peptide

MM is a commonly encountered hematological malignancy with significant renal involvement, and it often presents as renal failure. Renal involvement in patients with MM complicates treatment and shortens life span. The main renal lesion observed is tubulointerstitial disease characterized by fibrosis and is frequently associated with light-chain-containing casts. Pituitary adenylate-cyclase-activating polypeptide-38 (PACAP38) has emerged as a promising novel therapy for preserving the kidney function in

Table 2

Adjuvants used with peptide vaccines in MM.

Adjuvants	Combined with peptide (antigen)	Mechanism	Trials type	Researchers
GM-CSF and IL-2	Idiotype antigen	Involve natural killer cells and CD8+ T cells	Balb/c mice	[36]
GM-CSF and IL-12	Idiotype antigen	Induce antitumor immunity; necessary for the generation of a Th1 response and for the augmentation of CTL activity	Patients	[30]
Phosphostim and IL-2	Idiotype antigen	Th2 (IL-5) response	Patients	[38]
	Non-peptidic antigens	To enable the selective outgrowth of $\gamma\delta$ T cells; trigger a transient but large expansion of circulating V γ 9V δ 2 T cells	Patients, cell lines and healthy donors	[39]
CPG and IL-2	Amino acids 180–188 of the TRP-2 protein Amino acids 257–264 of the ovalbumin protein Amino acids 366–374 of the influenza A nucleoprotein Amino acids 49–57 of the human papillomavirus E7 Protein Amino acids 128–140 of the hepatitis B coreprotein	Increase the size of CD8+ T cell responses	C57BL/6 mice and cell lines	[40]
5-Azacytidine	SEMG I	Unclear	Patients and cell lines	[41]
IL-4 and IL-6	SEMG I	Enhance SEMG I expression through increasing its promoter function.	Patients and cell lines	[41]

patients with MM. As a pituitary immunomodulating peptide, PACAP38 can prevent light-chain-induced renal injury both *in vitro* and *in vivo* [35].

3. Advanced approaches for enhancing the immune response

Anticancer immunotherapy embodies the ideal antitumor therapy, as it theoretically combines target specificity with long-term disease control. The use of synthetic peptides as vaccines aimed at the induction of therapeutic responses against MM cells initially experienced great enthusiasm, mostly because of advances in vaccine technology, including design, synthesis, and delivery. Nevertheless, the application of such vaccines in humans has had limited success. In this process, there are many obvious shortcomings: (1) a vaccine's short half-life makes its APCs presentation by MHC class in a manner restricted difficult, which means the Th1 reaction and CTL protective immunogenic responses elicited are not as effective as expected; (2) TAAs exhibit autoimmunity responses. Most of the human TAAs characterized thus far are derived from nonmutated “self”-proteins. Numerous strategies have been developed to inhibit tolerance to TAAs, combining various forms of antigens with different vectors and adjuvants. However, few studies have determined how to select epitopes within a given TAA to avoid the autoimmunity response in MM. Despite this strong rationale, the promising preclinical results and the frequent induction of antigen-specific immune responses, peptide-based cancer vaccines have yielded relatively poor results in the clinical setting, a phenomenon likely due to the immunosuppressive properties of the tumor microenvironment that allow malignant cells to evade both naturally occurring and therapeutically induced immune surveillance. The clear identification of precise molecular characteristics of epitopes would help define the basic mechanisms of the self/non-self-distinction and lead to a greater understanding of phenomena such as tolerance, autoimmunity, and tumor escape of immune surveillance. Thus, the selection and modification strategies of antigen epitopes are key to the development of therapeutic vaccines. To enhance the immune

response, several approaches are being investigated as novel treatment modalities for MM.

3.1. Adjuvants

Adjuvants in MM immunology are often used to modify or augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, and, thus, providing increased immunity in an attempt to improve antitumoral potential (Table 2). For example, therapeutic effects of idiotype vaccination can be enhanced by combining granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2 in a myeloma model [36]. Hansson et al. [37] reported on 28 patients with IgG myeloma stages I to II who were immunized i.d. over 110 weeks with autologous M-protein combined with IL-12 versus with IL-12 and GM-CSF. Idiotype-specific immune responses were noted in 5 of 15 (33%) patients in the IL-12 group and in 11 of 13 (85%) patients in the GM-CSF/IL-12 group ($P < 0.01$). Moreover, the absence of an immune response was associated with an increase in the numbers of regulatory T cells. Abdalla et al. [38] investigated anti-idiotypic T cells in 18 MM patients vaccinated with idiotype protein plus the adjuvant cytokines GM-CSF and/or IL-12. Idiotype-specific T cells developed in 78% of those given IL-12/GM-CSF, but only in 22% of those given only IL-12. A Th1 (IFN- γ /TNF- α) immune response predominated in the IL-12 group while a Th2 (IL-5) response prevailed in the IL-12/GM-CSF group. Burjanadzé et al. [39] found that a 14-day culture of PBMCs with Phosphostim and IL-2 triggered a 100-fold expansion of gamma-delta T cells in 78% of newly diagnosed MM patients. Expanded gamma-delta T cells killed 13 of 14 myeloma cell lines as well as primary myeloma cells, but not normal CD34 cells, demonstrating the ability of Phosphostim (a synthetic phosphoantigen) and IL-2 to expand gamma-delta T cells obtained from MM patients, and this therapy can efficiently and stably kill human myeloma cells. Kochenderfer et al. [40] found that low-dose subcutaneous IL-2 dramatically increased the size of splenic and PB epitope-specific CD8(+) T-cell responses generated by peptide plus CpG-containing vaccines. Moreover, combining peptide plus CpG-containing vaccines with low-dose IL-2 mediated antitumor

Table 3
Present servers for analyzing T-cell epitopes in MM.

Server name	URL	Function	Researchers
SYFPEITHI	http://www.syfpeithi.de	Database for MHC ligands and peptide motifs.	[17] [19] [27] [42] [17]
BIMAS	http://www-bimas.cit.nih.gov	Rank potential 8-mer, 9-mer, or 10-mer peptides based on a predicted half-time of dissociation to HLA-class-I molecules.	[15] [19] [27] [32] [42]
LPpep	http://zlab.bu.edu/zhiping/lppep.html	Prediction of high affinity HLA-A2-binding peptides.	[42]
MHCPred	http://193.133.255.13/mhcpred/	Binding affinity calculation for heteroclitic peptides.	[19]
NetMHC	http://www.cbs.dtu.dk/services/NetMHC-2.0	Predicts binding of peptides to a number of different HLA alleles using artificial neural networks and hidden Markov models.	[10]
IEDB	http://www.immuneepitope.org http://tools.immuneepitope.org/analyze/cgi-bin/mhc.I.binding.py	MHC-I-binding predictions.	[49]
ProPred	http://www.imtech.res.in/raghava/propred	Predict MHC class-II-binding regions.	[44]
PePSSI	Non-display	Flexible structural prediction of peptide binding to MHC molecules.	

immunity. Indeed, there is no doubt that useful adjuvants need to be developed and incorporated into future vaccines. Moreover, it is recognized that vaccines designed for certain indications require a matching combination of selected antigen together with a critical immunopotentiator.

Semenogelin (SEMG) I is a potential candidate protein for MM vaccines because the aberrant expression of its gene has only been found in hematologic malignancies including MM. However, one major problem preventing effective clinical tumor immunotherapy is that the expression frequency in MM is less than 10%. To solve this problem, Zhang et al. [41] found that SEMG I expression in myeloma cells could be upregulated by 5-azacytidine, IL-4, and IL-6. The mechanisms of SEMG I gene upregulation by 5-azacytidine are unclear because there was no correlation between the methylation of the single CpG dinucleotide at position −11 and SEMG I expression. However, both IL-4 and IL-6 appeared to enhance SEMG I expression by enhancing its promoter function.

3.2. Bioinformatics tools

Immune bioinformatics and the development of computer algorithms to identify dominant T-cell epitopes based on sequence data provide bioinformatics tools of inestimable value in predicting the structure and function of new genes, which directs experimental efforts toward the most promising peptides. Various methods are used to analyze the epitopes of proteins, and peptides are identified when designing a vaccine for a target MM population (Table 3). For example, WT1-specific CTL epitopes with a restriction of HLA-A have already been identified by the NetMHC 2.0 Server prediction program [5]. HM1.24, PAK2, CDKN1A, SPAN-Xb, and IgGH gene-derived peptides with putative strong binding to HLA-A2 were identified using two independent algorithms for predicting HLA peptide binding (“SYFPEITHI” and “BIMAS”) in *in silico* analyses [17,19,27,30]. HLA-A*0201-restricted DKK1 and HLA-A24-restricted Hsp70 epitopes with strong affinities were identified only using the BIMAS algorithm [15,31]. Kondo et al. [42] used multiple computer algorithms to predict HLA-A*0201-

binding cyclin-D1 epitopes and found that HLA-A*0201-matched, primary cyclin-D1(+) tumor cells were efficiently recognized by cyclin-D1-specific CTLs. McMahan and Slansky [43] confirmed that TAA-derived mimotopes could either enhance binding to the MHC or increase the number of TCR-peptide-MHC complexes. Furthermore, it is difficult for endogenous antigens to activate mimotope vaccines. McMahan et al. also illustrated that mimotope vaccines elicit larger immunological responses in tumor cells than in normal cells. Schiewe and Haworth [44] recently developed an algorithm called PePSSI for the flexible structural prediction of peptide binding to MHC molecules, and they applied this algorithm to identify peptide epitopes from the CT-antigen sequence based on the potential of these peptides to bind to the human MHC molecule HLA-A2. This algorithm may provide a new method for identifying more T-cell epitopes for peptide vaccines.

3.3. Modified epitope peptide

Optimized peptides produced by modifying a single amino-acid substitution, PMRI, and lipid-modified peptides or peptide epitope fusion protein can efficiently enter APCs, improve their affinity with the MHC, greatly increase T-cell receptor binding or triggering sites, and enhance resistance to serum peptidase [45]. As described above, the modifying SPAN-XB-derived peptides can elicit more consistent CD8(+) T-cell responses in both healthy donors and patients with MM [27]. May et al. [46] embedded a synthetic immunogenic analog CD8(+) epitope (aa 126–134) inside the longer peptide (aa 122–140), by mutating residue 126 from an arginine to a tyrosine, and produced WT1 peptides (aa 328–349 and 423–441) as well as WT1 CD4(+) (aa 122–140). Their results showed that the former two epitopes are able to stimulate a peptide-specific CD4(+) response that can recognize WT1(+) tumor cells in multiple HLA-DRB1 settings. In contrast, the mutated WT1 peptide epitope (aa 122–140) is able to induce CD4(+) and cytotoxic CD8(+) WT1-specific T-cell responses that can recognize native WT1 epitopes on the surface of human WT1(+) cancer cells. These studies provide the rationale for using WT1 CD4(+) peptides in conjunction with CD8(+)

peptide epitopes to vaccinate patients with WT1-expressing cancers.

Analog heteroclitic peptides with increased immunogenicity can be synthesized and are potential cancer vaccine candidates, such as Melan-A [18] and WT1 [46]. Pinilla-Ibarz et al. [47] improved human T-cell responses against synthetic HLA-A*0201 analog peptides derived from the WT1 oncoprotein. Their strategy for breaking tolerance involved designing synthetic immunogenic analog peptides that could cross-react with the native peptides. Several synthetic peptides derived from the nonamer sequences of the WT1 protein were designed in which single amino-acid substitutions were introduced at HLA-A*0201 MHC-binding positions. Several of these new peptides could stabilize MHC class-I A*0201 molecules better than native sequences. Some analogs were also able to elicit WT1-specific T-cell recognition and CTLs more effectively than native sequences.

3.4. Microspheres

Microspheres have been shown to be an effective vaccine delivery technology for eliciting comprehensive immune responses and CD8(+) T-cell responses in particular. With the ultimate goal of developing a therapeutic cancer vaccine, Mossman et al. [48] encapsulated the Her-2/neu peptide p369–377 in poly(lactic-co-glycolide) microspheres. This formulation was found to effectively elicit CD8(+) CTL responses in an HLA-A*0201 transgenic mouse model. In contrast, immunization with either peptide alone or peptide formulated with an incomplete Freund's adjuvant failed to elicit such CTL responses. Responses induced by the peptide-microsphere formulation were enhanced by delivering increased doses of peptide and with repeated administrations over time. Coadministration of the peptide-microspheres with adjuvants, including GM-CSF, MPL adjuvant, and selected synthetic Toll-like receptor 4 ligands (the aminoalkyl glucosaminide-4 phosphates) significantly augmented CTL responses.

3.5. Adoptive therapy for tumor immunity

Dysfunction of the immune system is a major obstacle in MM treatment that can promote tumor growth and resistance to chemotherapy. Recent research has demonstrated the efficacy of combining peptide vaccines with adoptive therapies. Szmania et al. [49] addressed immunization of a healthy donor with MAGE-A3 peptide to induce immune responses that can be transferred and expanded after transplantation into the recipient, and can provide a new therapeutic option for high-risk MM.

3.6. Recombinant bacteriophages

Small synthetic peptides are poor antigens, and carrier proteins are important for improving the efficacy of synthetic peptide vaccines. Recombinant bacteriophages appear to be promising tools for designing subunit vaccines. Ulivieri et al. [50] reported that different HCMV MHC class-II-restricted peptides appeared on the capsid of filamentous bacteriophages, and they found that hybrid bacteriophages are processed by human APCs and activate HCMV-specific CD4 T cells. Using the filamentous bacteriophage as an epitope carrier, they detected that the response of the reporter T-cell hybridoma was more robust and longer lasting than that elicited by peptide stimulation.

3.7. Cell-penetrating peptides

A fundamental requirement for an effective vaccine is the ability to deliver antigen epitopes to APCs and the ensuing processing, presentation, and induction of an immune response. However,

vaccination with proteins or synthetic peptides incorporating CTL epitopes has been shown to limit effectiveness due to the failure of exogenous antigens to efficiently present to T cells. There has recently been a surge of interest in cell-penetrating peptides (CPP) as an efficient means of delivering therapeutic targets into cellular compartments [51]. Moreover, linking antigens to CPP overcomes such obstacles by facilitating the cellular uptake, processing, and presentation of exogenous antigens for inducing potent immune responses. It is clear that CPP fusion proteins are efficiently taken up by human monocyte-derived DC and are able to induce antigen-specific T cells.

3.8. Multiple antigen peptides

The development of new peptide drugs has been significantly restricted by their short half-life. Branched peptides, such as multiple antigen peptides (MAPs), were invented in the 1980s, and their ability to reproduce single epitopes for stimulating the immune system has been extensively tested in the field of new vaccine discovery. Pini et al. [52] discovered that MAP molecules acquire strong resistance to proteases and peptidases. This resistance renders MAPs very stable and, thus, suitable for drug development.

4. Concluding remarks

Thus far, many immunotherapeutics developed for MM directly deprive MM cells of their ability to selectively respond to proliferative and antiapoptotic microenvironmental cues. Specifically, the discovery of tumor antigens recognized by T cells and the features of immune responses directed against them has prompted a multitude of studies aimed at boosting antitumor T-cell immunity. In this respect, peptide-based immunotherapy is one potentially promising strategy for generating desirable immune responses and is rapidly changing the survival outlook [53]. Moreover, innovations mainly using adjuvants and bioinformatics appear to enhance peptide presentation and accessibility to the immune system and augment specific immune responses, which increase the likelihood of immune responses providing clinical benefits. Therefore, it is feasible to develop novel therapeutic approaches based on the epitopes, the minimal immunogenic region of an antigen. In the future, peptides may be utilized to make therapeutic agents that are specific, long lasting, and affordable, and that exhibit minimal toxicity.

Conflict of interest statement

None declared.

Acknowledgment

This project was supported by a grant from the National Natural Science Foundation of China (No. 30700337) for the research of MM.

References

- [1] Rosenblatt J, Avigan D. Cellular immunotherapy for multiple myeloma. *Best Pract Res Clin Haematol* 2008;21:559–77.
- [2] Kastritis E, Charidimou A, Varkaris A, Dimopoulos MA. Targeted therapies in multiple myeloma. *Target Oncol* 2009;4:23–36.
- [3] Perez SA, von Hofe E, Kallinteris NL, Gritzapis AD, Peoples GE, Papamichail M, et al. A new era in anticancer peptide vaccines. *Cancer* 2010;116:2071–80.
- [4] Tsuboi A, Oka Y, Nakajima H, Fukuda Y, Elisseeva OA, Yoshihara S, et al. Wilms tumor gene WT1 peptide-based immunotherapy induced a minimal response in a patient with advanced therapy-resistant multiple myeloma. *Int J Hematol* 2007;86:414–7.
- [5] Li Z, Oka Y, Tsuboi A, Fujiki F, Harada Y, Nakajima H, et al. Identification of a WT1 protein-derived peptide, WT1, as a HLA-A 0206-restricted, WT1-specific CTL epitope. *Microbiol Immunol* 2008;52:551–8.
- [6] Oka Y, Tsuboi A, Oji Y, Kawase I, Sugiyama H. WT1 peptide vaccine for the treatment of cancer. *Curr Opin Immunol* 2008;20:211–20.

- [7] Oka Y, Tsuboi A, Fujiki F, Li Z, Nakajima H, Hosen N, et al. WT1 peptide vaccine as a paradigm for “cancer antigen-derived peptide”-based immunotherapy for malignancies: successful induction of anti-cancer effect by vaccination with a single kind of WT1 peptide. *Anticancer Agents Med Chem* 2009;9:787–97.
- [8] Tang CK, Katsara M, Apostolopoulos V. Strategies used for MUC1 immunotherapy: human clinical studies. *Expert Rev Vaccines* 2008;7:963–75.
- [9] Brossart P, Schneider A, Dill P, Schammann T, Grünebach F, Wirths S, et al. The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T-lymphocytes. *Cancer Res* 2001;61:6846–50.
- [10] Choi C, Witzens M, Bucur M, Feuerer M, Sommerfeldt N, Trojan A, et al. Enrichment of functional CD8 memory T cells specific for MUC1 in bone marrow of patients with multiple myeloma. *Blood* 2005;105:2132–4.
- [11] Kryukov F, Ocadičková D, Kovárová L, Buresová I, Hájek R, Michálek J. In vitro activation of cytotoxic T-lymphocytes by hTERT-pulsed dendritic cells. *J Immunotoxicol* 2009;6:243–8.
- [12] Ocadičková D, Kovárová L, Hájek R, Michálek J. The preparation of myeloma-specific T cells activated with dendritic cells loaded with nonapeptides derived from mucin protein MUC1 and catalytic subunit of telomerase Htert. *Klin Onkol* 2008;21:59–65.
- [13] Schmitt M, Schmitt A, Rojewski MT, Chen J, Giannopoulos K, Fei F, et al. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. *Blood* 2008;111:1357–65.
- [14] Gavriatopoulou M, Dimopoulos MA, Christoulas D, Migkou M, Iakovaki M, Kkotzamanidou M, et al. Dickkopf-1: a suitable target for the management of myeloma bone disease. *Expert Opin Ther Targets* 2009;13:839–48.
- [15] Qian J, Xie J, Hong S, Yang J, Zhang L, Han X, et al. Dickkopf-1 (DKK1) is a widely expressed and potent tumor-associated antigen in multiple myeloma. *Blood* 2007;110:1587–94.
- [16] Grube M, Moritz S, Obermann EC, Rezvani K, Mackensen A, Andreesen R, et al. CD8+ T cells reactive to survivin antigen in patients with multiple myeloma. *Clin Cancer Res* 2007;13:1053–60.
- [17] Hundemer M, Schmidt S, Condomines M, Lupu A, Hose D, Moos M, et al. Identification of a new HLA-A2-restricted T-cell epitope within HM1.24 as immunotherapy target for multiple myeloma. *Exp Hematol* 2006;34:486–96.
- [18] Christensen O, Lupu A, Schmidt S, Condomines M, Belle S, Maier A, et al. Melan-A/MART1 analog peptide triggers anti-myeloma T-cells through crossreactivity with HM1.24. *J Immunother* 2009;32:613–21.
- [19] Li G, Hundemer M, Wolfrum S, Ho AD, Goldschmidt H, Witzens-Harig M. Identification and characterization of HLA-class-I-restricted T-cell epitopes in the putative tumor-associated antigens P21-activated serin kinase 2 (PAK2) and cyclin-dependent kinase inhibitor 1A (CDKN1A). *Ann Hematol* 2006;85:583–90.
- [20] Atanackovic D, Arfsten J, Cao Y, Gnjatich S, Schnieders F, Bartels K, et al. Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation. *Blood* 2007;109:1103–12.
- [21] Andrade VC, Vettore AL, Felix RS, Almeida MS, Carvalho J, Oliveira JS, et al. Prognostic impact of cancer/testis antigen expression in advanced stage multiple myeloma patients. *Cancer Immun* 2008;8:2.
- [22] Atanackovic D, Luetkens T, Hildebrandt Y, Arfsten J, Bartels K, Horn C, et al. Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma. *Clin Cancer Res* 2009;15:1343–52.
- [23] Tinguely M, Jenni B, Knights A, Lopes B, Korol D, Rousson V, et al. MAGE-C1/CT-7 expression in plasma cell myeloma: sub-cellular localization impacts on clinical outcome. *Cancer Sci* 2008;99:720–5.
- [24] Goodyear O, Piper K, Khan N, Starczynski J, Mahendra P, Pratt G, et al. CD8+ T cells specific for cancer germline gene antigens are found in many patients with multiple myeloma, and their frequency correlates with disease burden. *Blood* 2005;106:4217–24.
- [25] Goodyear OC, Pratt G, McLarnon A, Cook M, Piper K, Moss P. Differential pattern of CD4+ and CD8+ T-cell immunity to MAGE-A1/A2/A3 in patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma. *Blood* 2008;112:3362–72.
- [26] van Rhee F, Szmania SM, Zhan F, Gupta SK, Pomtree M, Lin P, et al. NY-ESO-1 is highly expressed in poor-prognosis multiple myeloma and induces spontaneous humoral and cellular immune responses. *Blood* 2005;105:3939–44.
- [27] Frank C, Hundemer M, Ho AD, Goldschmidt H, Witzens-Harig M. Cellular immune responses against the cancer-testis antigen SPAN-XB in healthy donors and patients with multiple myeloma. *Leuk Lymphoma* 2008;49:779–85.
- [28] Bogen B, Ruffini PA, Corthay A, Fredriksen AB, Frøyland M, Lundin K, et al. Idiotype-specific immunotherapy in multiple myeloma: suggestions for future directions of research. *Haematologica* 2006;91:941–8.
- [29] Cohen S, Haimovich J, Hollander N. B-cell lymphoma and myeloma protection induced by idiotype vaccination with dendritic cells is mediated entirely by T cells in mice. *J Immunother* 2005;28:461–6.
- [30] Hansson L, Rabbani H, Fagerberg J, Osterborg A, Mellstedt H. T-cell epitopes within the complementarity-determining and framework regions of the tumor-derived immunoglobulin heavy chain in multiple myeloma. *Blood* 2003;101:4930–6.
- [31] Belle S, Han F, Condomines M, Christensen O, Witzens-Harig M, Kasper B, et al. Identification of HLA-A2 restricted T-cell epitopes within the conserved region of the immunoglobulin G heavy-chain in patients with multiple myeloma. *Eur J Haematol* 2008;81:26–35.
- [32] Okochi M, Hayashi H, Ito A, Kato R, Tamura Y, Sato N, et al. Identification of HLA-A24-restricted epitopes with high affinities to Hsp70 using peptide arrays. *J Biosci Bioeng* 2008;105:198–203.
- [33] Gullo CA, Hwang WY, Poh CK, Au M, Cow G, Teoh G. Use of ultraviolet-light irradiated multiple myeloma cells as immunogens to generate tumor-specific cytolytic T lymphocytes. *J Immune Based Ther Vaccines* 2008;6:2.
- [34] Lotz C, Mutallib SA, Oehlrich N, Liewer U, Ferreira EA, Moos M, et al. Targeting positive regulatory domain 1-binding factor 1 and X box-binding protein 1 transcription factors by multiple myeloma-reactive CTL. *J Immunol* 2005;175:1301–9.
- [35] Sengul S, Li M, Batuman V. Myeloma kidney: toward its prevention—with new insights from in vitro and in vivo models of renal injury. *J Nephrol* 2009;22:17–28.
- [36] Stritzke J, Zunkel T, Steinmann J, Schmitz N, Uharek L, Zeis M. Therapeutic effects of idiotype vaccination can be enhanced by the combination of granulocyte-macrophage colony-stimulating factor and interleukin 2 in a myeloma model. *Br J Haematol* 2003;120:27–35.
- [37] Hansson L, Abdalla AO, Moshfegh A, Choudhury A, Rabbani H, Nilsson B, et al. Long-term idiotype vaccination combined with interleukin-12 (IL-12), or IL-12 and granulocyte macrophage colony-stimulating factor, in early-stage multiple myeloma patients. *Clin Cancer Res* 2007;13:1503–10.
- [38] Abdalla AO, Hansson L, Eriksson I, Näsman-Glaser B, Rossmann ED, Rabbani H, et al. Idiotype protein vaccination in combination with adjuvant cytokines in patients with multiple myeloma—evaluation of T-cell responses by different read-out systems. *Haematologica* 2007;92:110–4.
- [39] Burjanadzé M, Condomines M, Reme T, Quittet P, Latry P, Lugagne C, et al. In vitro expansion of gamma delta T cells with anti-myeloma cell activity by Phosphostim and IL-2 in patients with multiple myeloma. *Br J Haematol* 2007;139:206–16.
- [40] Kochenderfer JN, Chien CD, Simpson JL, Gress RE. Maximizing CD8+ T cell responses elicited by peptide vaccines containing CpG oligodeoxynucleotides. *Clin Immunol* 2007;124:119–30.
- [41] Zhang Y, Wang Z, Zhang J, Farmer B, Lim SH. Semenogelin I expression in myeloma cells can be upregulated pharmacologically. *Leuk Res* 2008;32:1889–94.
- [42] Kondo E, Maecker B, Weihrauch MR, Wickenhauser C, Zeng W, Nadler LM, et al. Cyclin D1-specific cytotoxic T lymphocytes are present in the repertoire of cancer patients: implications for cancer immunotherapy. *Clin Cancer Res* 2008;14:6574–9.
- [43] McMahan RH, Slansky JE. Mobilizing the low-avidity T cell repertoire to kill tumors. *Semin Cancer Biol* 2007;17:317–29.
- [44] Schiewe AJ, Haworth IS. Structure-based prediction of MHC-peptide association: algorithm comparison and application to cancer vaccine design. *J Mol Graph Model* 2007;26:667–75.
- [45] Iero M, Filipazzi P, Castelli C, Belli F, Valdagni R, Parmiani G, et al. Modified peptides in anti-cancer vaccines: are we eventually improving anti-tumour immunity? *Cancer Immunol Immunother* 2009;58:1159–67.
- [46] May RJ, Dao T, Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Zhang RH, et al. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clin Cancer Res* 2007;13:4547–55.
- [47] Pinilla-Ibarz J, May RJ, Korontsvit T, Gomez M, Kappel B, Zakhaleva V, et al. Improved human T-cell responses against synthetic HLA-0201 analog peptides derived from the WT1 oncoprotein. *Leukemia* 2006;20:2025–33.
- [48] Mossman SP, Evans LS, Fang H, Staas J, Tice T, Raychaudhuri S, et al. Development of a CTL vaccine for Her-2/neu using peptide-microspheres and adjuvants. *Vaccine* 2005;23:3545–54.
- [49] Szmania S, Gnjatich S, Tricot G, Stone K, Zhan F, Moreno A, et al. Immunization with a recombinant MAGE-A3 protein after high-dose therapy for myeloma. *J Immunother* 2007;30:847–54.
- [50] Olivieri C, Citro A, Ivaldi F, Mascolo D, Ghittoni R, Fanigliulo D, et al. Antigenic properties of HCMV peptides displayed by filamentous bacteriophages vs. synthetic peptides. *Immunol Lett* 2008;119:62–70.
- [51] Brooks NA, Pouniotis DS, Tang CK, Apostolopoulos V, Pietersz GA. Cell-penetrating peptides: application in vaccine delivery. *Biochim Biophys Acta* 2010;1805:25–34.
- [52] Pini A, Falciani C, Bracci L. Branched peptides as therapeutics. *Curr Protein Pept Sci* 2008;9:468–77.
- [53] Mocellin S, Pilati P, Nitti D. Peptide-based anticancer vaccines: recent advances and future perspectives. *Curr Med Chem* 2009;16:4779–96.