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Abstract

Colorectal cancer (CRC) is one of the most common cancers globally and is associated with a high rate of morbidity and mortality. A large proportion of patients with early stage CRC who undergo conventional treatments develop local recurrence or distant metastasis and in this group of advanced disease, the survival rate is low. Furthermore there is often a poor response and/or toxicity associated with chemotherapy and chemo-resistance may limit continuing conventional treatment alone. Choosing novel and targeted therapeutic approaches based on clinicopathological and molecular features of tumors in combination with conventional therapeutic approach could be used to eradicate residual micrometastasis and therefore improve patient prognosis and also be used preventively. Peptide-based vaccination therapy is one class of cancer treatment that could be used to induce tumor-specific immune responses, through the recognition of specific antigen-derived peptides in tumor cells, and this has emerged as a promising anti-cancer therapeutic strategy. The aim of this review was to summarize the main findings of recent studies in exciting field of peptide-based vaccination therapy in CRC patients as a novel therapeutic approach in treatment of CRC.

Keywords: Peptide, vaccine, immunotherapy, colorectal cancer, treatment

Introduction

Colorectal cancer (CRC) is one of the most common cancers globally, and accounts for a high rate of morbidity and mortality (1), and can severely impair quality of life (2). It is accounted that annually greater than one million new cases are diagnosed world-wide (3). The CRC incidence rate varies geographically, however in men and women are affected nearly equal (4, 5). In some regions like United States, several European countries, Canada, Australia, and New Zealand the incidence rate is high. In contrast, in East and central Asia and some parts of Africa and South America it has been observed to have low incidence rate (4, 6). Positive family history, presence of some specific genetic properties, age, obesity, inflammatory bowel disease and over-consumption of alcohol and red meat are considered to be an important risk factors for CRC (7-9). The CRC incidence and its related mortality has started to decrease in high-income countries and also in individuals over the age of fifty, however this has continued to increase in low-income countries and under the age of 50 (10). The patient survival rate is highly dependent on the stage at diagnosis and treatment, being approximately 90% in patients who undergo treatment in the early stages (stage I and II) whilst roughly 70% in patients with regional lymph node metastasis and less than 10% in distant metastatic disease (3, 11). CRC patients are commonly treated with similar chemoradiotherapeutic and surgical protocol (12). Despite many advances that have occurred during the last few years in the detection and successful therapeutic approach in early stage, a large proportion of CRC patients diagnosed and treated in the early stages ultimately develop recurrence or distant metastasis (13, 14). Furthermore the poor response to chemotherapy, and the toxicity and chemo-resistant

cancer has limited the benefits of conventional treatment alone. With respect to variation in clinicopathological and molecular features of tumors between CRC patients to patients, choosing novel and specific therapeutic approaches based on these differences in combination with conventional therapeutic approach could eradicate residual micrometastasis and therefore improve patient prognosis and also serving as a preventive measure. Recently immunotherapy using cancer vaccines have entered trials in a variety of cancers as a promising anti-cancer therapeutic strategy and has shown impressive clinical benefit due to being well tolerated and being without dose-limiting toxicities (15, 16).

Peptide-based vaccination therapy is one type of cancer vaccines which has been investigated in CRC treatment (17). This form of vaccine induce tumor-specific T cell mediated immune responses through recognition of specific antigen-derived peptides in tumor cells (17). Regarding recent advances in the genetic based research, personalized medicine is a growing field which is findings its place within the vast majority of developing sciences. In cancer research, there have been benefits from this new approach to personalized medicine in term of treatment and diagnosis. Personalized vaccine therapy is an emerging field in cancer treatment. In this therapy, according to recent status of patient's immune system and some other individual tumor factors, a more specific and potent vaccine is developed for specific individuals (18) (Figure 1). The aim of this review is to summarize the main findings of recent studies in exciting field of peptide-based vaccination therapy in CRC patients as a novel therapeutic approach in treatment of CRC.

Conventional treatment of colorectal cancer

Treatment of colorectal cancer relies on disease stage and involves a multidisciplinary approach (2, 19, 20). Treatment depends on three main approaches: surgery, chemotherapy and radiotherapy. Surgical treatment is the main curative treatment in non-metastasized CRC patients (stage I and II) (21). During surgical treatment; in addition to tumoral tissue removal, a small margin of surrounding tumor free tissue and adjacent lymph node are removed (22). Based on several conditions including patient's general health, surgeon's experience and available equipment in surgical center, tumor resection can be done by laparoscopic resection or open surgery. Preoperative chemotherapy and/or radiotherapy, also called neoadjuvant treatment, can decrease tumor size and local recurrence and increase chance of successful tumor resection (23). Adjuvant treatment, also known as postoperative chemoradiotherapy, improves survival after surgical treatment (24). The most common protocol used in chemotherapy is the FOLFOX protocol (consists 5-Fluorouracil, Folinic Acid (Leucovorin) and Oxaliplatin) and CapeOx protocol (consists capecitabine and oxaliplatin) (24). When tumoral cells have spread to regional lymph nodes (CRC stage III) partial colectomy is usually accompanied by surgical removal of metastatic lymph nodes. Furthermore, in these patients adjuvant chemotherapy can improve outcomes (2). In CRC stage IV in which cancerous cells are involve distant organs and lymph nodes, surgery in most patients is not possible, however it can be effective if both the primary tumor and distant metastases are small and resectable (25). In these patients, chemoradiotherapy is the mainstay therapy, to reduce symptoms and prolong survival.

With the aim of improving survival, conventional therapies could be combined with immunotherapy which is less toxic, well-tolerated and more effective therapeutic approach to eradicate residual CRC cells especially in metastatic CRC.

Cancer vaccines as a novel immunotherapeutic approach in colorectal cancer

The early consideration of using cancer vaccines was reported in late 19th when the first allogeneic melanoma lysates were used as cancer vaccine (26). Later on, researchers have focused on designing more specific and effective vaccines for many other types of cancers and in 2010, the first cancer vaccine was approved by the USA's Federal Drug Administration for prostate cancer (27). The rapid improvement of developing different types of vaccines is still ongoing and new types of genetic vaccines such as neoantigen based vaccines are under active research in clinical trials (28). According to available the strategies for developing cancer vaccines, cancer vaccine can be categorized into 3 main types. Dendritic cell vaccines (DCVs) are considered as an efficient approach among cancer vaccines. DCVs essentially use the human immune system to fight against cancers. During this approach, dendritic cells (DC) which are one of the antigen presenting cells and T lymphocytes which are responsible for cell-mediated immunity will become involved (29). After a leukapheresis, DCs are maturated and presented with a specific tumor antigen ex vivo. Then these antigen-plused DCs are returned to the patient and present their tumor antigen to T lymphocytes (29). Allogeneic whole tumor cell lysate have been used in human CRC and achieved notable success. Toh et al. have loaded DCs with melanoma cell lysate which were

expressing melanoma-associated antigen gene and delivered them to advanced CRC patients (30). Using the DCV approach results in boosting the human immune system response against tumor cells. In order to increasing the efficacy and overcoming the limitation of DCVs, researchers have tried different approaches. The main drawback of these types of vaccines which has to be overcome is the need for long term culturing DCs which was solved by using different DC subtypes. Also, by using neoantigens and whole tumor antigens, they could achieve a more favorable immune response against tumor antigens (31). The second type of cancer vaccines are termed as genetic vaccines (28). The major advantage of this type of vaccines in contrast to DCVs is the ability of delivering multiple types of antigens to a desired cell (32). During this method, plasmid DNA vectors which carry a desired antigen sequence are transferred to human cells which are mostly DCs. Expression of these gene cassettes results in the presentation of antigens to the immune system (32). DNA vaccines has been previously applied in colorectal cancer. A plasmid encoding the carcinoembryonic antigen (CEA) gene was given along with cyclophosphamide intramuscularly or intradermaly. The vaccine was well tolerated and only minor adverse events such as myalgia, fatigue and headache was reported (33). The major limitation of DNA vaccines is some problems with their efficacy and safety in contrast to DCVs. The expression of the desired cassette is not always achieved and fusion of the DNA into the human genome may be a limiting issues (34). mRNA vaccines have resolved some of these limitations but achieving a stable and long lasting RNA is remains a problem (35). Researchers have also used viruses as a possible cancer vaccine (36). Viruses can act as both vectors and oncolytics. However, these viral assistants still leave a safety issue which was

previously noted with respect to DNA vaccines (37). The third group of cancer vaccines are peptide vaccines. This type of vaccine uses tumor associated antigen peptides to activate immune system. Single and multiple peptide vaccines have been used in cancer patients. The personalized peptide vaccine (PPV) is a new way in treating cancer. During this type of vaccination, there is a rapid and strong secondary immune response that develops according to pre-existing immunity in a cancer patient. Table 1 has summarized the main differences between using personalized vaccines and common vaccines.

The mechanism of anti-tumor effect of peptide-based vaccination therapy

Traditional vaccination is usually performed by injecting a whole pathogen or its cellular membrane into a human body intramuscularly, subcutaneously or even by gastro intestinal tract such as oral Polio vaccines. Although peptide vaccination shares a similar approach as traditional vaccines, there are some special characteristics that have made it a potent vaccine even in treating non communicable diseases such as cancer. Regardless of vaccine type, almost all types of vaccines affect the human immune system. Tumor cells have antigens that are shared with viruses or bacterial pathogens which have potential to be used as vaccines. Antigen presenting cell in human circulating system uptake and process these protein antigens (38). These processed peptides will be presented to other immune system's cells; lymphocytes. T cell lymphocytes can be a cytotoxic T lymphocyte (CD8+) or helper T lymphocytes (CD4+). The cytotoxic T cells have the ability to recognize and directly attack tumor cells. These cells have the ability to kill tumor cells through inducing apoptosis,

releasing perforins and geransymes (39). T helper lymphocytes can release cytokines which are used to recruit more immune cells, especially natural killer cells (Figure 2).

The peptides used as vaccines act in a similar manner. These peptides are mostly unique tumor associated antigens (TAAs). TAAs which are result of cancerous mutations are potentially a strong immune system activators in most tumors (40). However, the rapid changes in tumor cell populations and the development of new mutations because of altered repair mechanisms in tumor cells, make it possible to introduce new TAA with less immunogenicity. The best approach in choosing a TAA, is choosing the most immunogenic forms. The more specific the selected epitope, the more specific the result would be. Choosing the best epitopes should also be directed toward the desired immune response from even cytotoxic T or B cell lymphocytes with special focus on enhancing helper lymphocytes function as well. The peptide length is also important. It has been reported that longer peptides are more likely to induce a greater immunogenic response (41). To achieve the most desirable and specific peptide antigens and avoiding shared tumor specific antigens, studying the main proteins which are important in development and survival of CRC cell is a crucial issue. Many proteins are considered as good targets for making a potent peptide vaccine in CRC patients. One of these proteins is Survivin. Survivin which is an inhibitor of tumoral cell apoptosis, is a target which is used in a study conducted by Tsuruma and colleagues. This variant can bind to HLA-A24 and is considered to be safe (42). Some studies have used another common protein which is seen in CRC (43-45). They have targeted SART, a tumor rejection antigen. Targeting this protein has been shown to have meaningful correlation with clinical outcomes as well as inducing immune system response (43-45).

Nowadays, choosing the best peptide has become easier and more cost-effective by use of immunoproteomics and in-silico technologies instead of experimental approaches (46). These bioinformatics tools are able to predict many features of desired peptide including the binding of peptide to MHC I and MHC II or even T cell receptors. Despite of using these in-silico models, the next step after synthesis of peptides would be to undertake preclinical tests on animal models. However, as is the case for other pathogens which enters human body and fight for living, tumor cells also have their own way for escaping immune response and result in failure of vaccination. Tumor cells can counter-attack cytotoxic lymphocytes by presenting FasL and induce lymphocytes to undergo apoptosis. Also, some tumor cells have the ability to reduce MHC expression and escape from recognition by immune system. Another mechanism which depends on tumor oncogenic pathways is production and releasing interleukins which are known as an inhibitor of immune system such as IL-10. Overcoming these issues is the cornerstone of developing a peptide vaccine. The main function of a potent vaccine is the appropriate stimulation of human immune response against tumors. In order to achieve this goal, determination of immune dominant domain epitopes is the first step of choosing the best peptide vaccine. These domains should have the ability to stimulate humeral or cellular immune system. Manipulating these peptides, in order to improve their affinity for MHCs, is mandatory to achieve better responses. Important factors for increasing the clinical response is summarized in table 2 and next chapter. Responding to peptide vaccines are HLA specific, which means that only those individual who express specific type of MHC will benefit from the vaccine and the risk of developing autoimmunity is still present when talking about using self-antigens.

Adjuvant and antigen delivery system in cancer vaccination therapy

Since peptide alone is usually insufficient to stimulate immune system to induce effective anti-tumor response, adjuvants are critical and required components of successful peptide-based vaccine strategies (47). A variety of adjuvants have been used in trials in treatment of various cancers. With the aim of enhancing immunogenicity and increasing anti-cancer immune responses adjuvants is administered with peptide in cancer vaccination (47). These adjuvants could be able to provide antigenic peptide protection from degradation and resulted in extended antigen presentation and better concentration, as well as an optimized availability of peptide and also higher expression of cytokines and co-stimulatory molecules by antigen presenting cells (APCs) (48, 49). As shown in figure 1, adjuvant which have been used in immunotherapy include cytokines such as interleukin-2 (IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon (IFN), antibodies such as programmed cell death protein-1 antibody (PD-1 Ab) and cytotoxic T-lymphocyte- associated protein-4 antibody (CTLA-4 Ab), agonists of stimulator of interferon genes (STING) and adjuvants targeting toll-like receptors (TLRs) like monophospholipid A (MPLA), imiquimod, CpG, Pam_3CSK_4 and Hiltonol (known as poly-ICLC). TLRs influence their downstream signaling cascades, and play an important role in the immune system response (50, 51). Pam_3CSK_4 is a synthetic lipopeptide that function as a TLR2 agonist whilst Hiltonol, MPLA, imiquimod,

and CpG activate TLR3, TLR4, TLR7/8 and TLR9, respectively (47). Based on peptide, cancer type, injection method, appropriate adjuvant is selected and there is not enough evidences that which adjuvant is superior to another. Of notice adjuvants may encompass desired and undesired properties. Therefore accumulated evidence of preclinical studies suggests that combining various adjuvants is provided more effective anti-tumor reaction. One possible reason for the wide differences in the reported benefit of anti-cancer vaccination in animal and human model of studies, may be the selection of safe and weak adjuvants in clinical trials in contrast to using potent adjuvants in animal models. On the other hand, antigen delivery systems which is also called class I adjuvants, besides activating the innate immune cells, through influencing several mechanisms promote antigen presentation to lymphocyte T cells and also increase antigen presentation time. Antigen delivery systems consist of incomplete Freund's adjuvant (IFA), or a Montanide formulation of IFA, micro/nano particles and aluminum. IFA is a water-in-oil emulsion that due to provide sustained release of emulsified antigen could be able to induce potent cellular response and antibody protection (47). Aluminum is the safe and the most commonly used adjuvant and both types, aluminum phosphate and aluminum hydroxide, are used in human vaccines (47, 52). The main role of alum in vaccination can be considered as a gradual releaser of vaccine at the vaccination site. Moreover, this adjuvant promotes T helper-2 immune responses. Recent studies has shown that using alum in combination with other adjuvants can provide better and mixed T helper 1 and 2 response(53). Aluminum provides an antigen depot and activation of inflammasome in dendritic cells resulted in production of proinflammatory cytokines which enhances immune responses (54). Micro/nano particles could be able

to deliver biologically active cargo into specific cell types. In addition, these small-sized particles protect their entities from degrading factors in serum or tissues and this resulted in decreased required antigen dose (55, 56). However, rapid clearance in plasma and the need to be combined with other adjuvants are important disadvantages of these small-sized particles (47). The type of induced response may be depend on particle size (57). Based on antigen, adjuvant and desired response micro/nano particles are produced from different natural or synthetic materials including collagen, gelatin, chitosan, poly(lactide-co-glycolide), polystyrene and poly(lactic acid) (47).

Role of personalized peptide-based vaccination therapy in colorectal cancer

Regarding the recent advances in personalized medicine, cancer therapy has also benefited from personalized approaches. Developing a personalized peptide vaccine (PPV) is almost the same as discussed above. However, there are some important steps prior to vaccine development. During the development of a PPV, pre-existing immunity to TAAs is the most important issue. In order to achieve this goal, individual's serum will be tested for different immunogenic MHC I epitopes from different TAAs. The patient's vaccine will be mainly based on one to four of these peptides (11). The reason behind choosing multiple antigen is the unavailability of most of tumors for characterization before treatment. Choosing more specific antigens will guarantee a better response (18). Kibe et al. has conducted a phase II clinical trial on PPV in CRC patients. They have selected four HLA matched antigens which were individually selected from a pool of candidate peptides. They have reported a favorable outcomes

and survival benefits in patients with advanced CRC who have been previously failed their treatment (43). While not all of their patients responded well to therapy, they tried to introduce a prognostic and predictive biomarker for their patients. They have reported that lower B-cell activating factor and higher IL-6, interferon gamma inducible protein-10 level prior to vaccination is related to worse survival. Sato has also conducted a similar study on CRC patients (58). They have chosen 4 peptides among their candidate panel of antigens applicable for vaccination and administered them along with 5-fluorouracil derivative. They concluded that 80mg/m^2/day of 5-fluorouracil derivative in combination with the vaccine will successfully augment cytotoxic T lymphocyte activity (58). Another study by Hattori et al. evaluated the effect of PPV and UFT and UZEL in metastatic CRC. They have prepared 25 and 23 peptides for HLA-A24+ and HLA-A2+ patients respectively. These peptides were identified by reverse immunology techniques or by cloned TAA genes. None of their patient's showed complete or even partial response and only 6 out of 14 patients had stable disease. However, combination of peptide vaccine and, UFT and UZEL was well tolerated and provided humeral and cellular response (59). Rahma et al. have also conducted three arms clinical study on 38 CRC patients and used mutant ras vaccine as a PPV (60). These peptides has the potentiality of inducing specific immune response along with adjuvants. They have suggested that using IL-2 as adjuvant may have negative effect on immune response induced by ras peptide vaccination (60). According to available results and hopeful results from phase I and II clinical trials, still we face lack of enough evidence to conclude the effectiveness PPV in improving the survival and more phase III clinical trials are warranted.

Conclusion and future prospective

There is a growing trend toward developing new cancer treatment and the vaccines seems to be an attractive approach for immunologists, oncologists, molecular geneticists and molecular engineering. Constructing different types of cancer vaccines, especially PPVs, requires a multidisciplinary team and a good research background for providing the best pool antigens. Recently, there are a growing number of completed phase I and II clinical trials for PPVs in CRC patients mostly with advanced and metastatic tumors. However, lack of phase III clinical trials is still highlighted in the literature. It also seems that some important issues are neglected in the literature which should be addressed in further researches. The most important topic stands for the interoperation of individual study results. In most of the studies it is still unclear why some patients don't respond well to PPVs while the others do so. More focusing on specific personalized differences in term of immune system genetic differences such as possible polymorphism or mutations may provide some answers for individual responses to same PPVs. Also, the role of microRNAs as an important prognostic and predictive markers in CRC is not evaluated in personalized response to PPVs. Researchers should also pay attentions to combination therapy of PPVs and newer therapeutic biomarkers such as targeting tumor microenvironment (61, 62). It's not clear even combination of different adjuvants can provide better responses in those patients who did not responded to PPVs. Another issue which should be addressed is prophylactic vaccination in CRC patients. While most of the studies have focused on advanced and poor responding CRC, it is not clear whether PPV vaccinations is helpful

in slowing the progression of CRC or inhibiting some CRC phenotypes such as colon polyps.

Figure 1. Adjuvants (blue circle) and delivery systems (yellow circle) for improvement of cancer vaccines efficacy

Figure 2. Peptide-based and personalized peptide-based vaccination therapy. The peptide antigen is first uptake by dendritic cells and the processed protein will be presented to immune system cells including cytotoxic T cells. After activation, these cells will kill tumor cells by inducing apoptosis and producing specific chemokine. During personalized base peptide vaccine therapy, a more specific response is expected. After mutation determination in tumoral patient, successful HLA typing is mandatory for producing an effective vaccine. The most effective peptide can be chosen by use of different software which are able to predict the efficacy of HLA-Peptide-TCR complex. Vast number of adjuvants are available for boosting the effect of personalized based vaccines which can be used along with vaccination. The immune monitoring will guarantee the efficacy of desired peptide vaccine in each individual.

Table 1. Personalized peptide vaccine in contrast to other common vaccines.

Vaccine type	Advantages	Disadvantages
Traditional peptide vaccine	Inexpensive and easy to produce Shorter preparation time Reduced chance of adverse immune reaction cytotoxic T lymphocyte induction	Slow immune response No memory T cell Induction of primary immune response
Personalized peptide vaccine	Faster and stronger response cytotoxic T lymphocyte induction Memory T cell activation Not inducing primary immune response	Expensive and more time consuming Longer preparation time Not inducing regulatory T cells

Table 2. Summary of the most relevant studies investigating peptide-based and personalized peptide-based vaccination therapy in colorectal cancer

Author and year	Study phase	Peptide vaccine	Adjuvant	Sample	Main side effect(s)	Main finding
Goydos et al., 1996 (63)	Phase I	mucin MUC-1	BCG	30 CRC	Injection-site reaction, fever	Profound delayed-type hypersensitivity to peptide vaccine observed and the vaccine was tolerated well. However they have failed to achieve tumor response in their trial.
Gonzalez et al., 1998 (64)	-	hu-EGF	Aluminium hydroxyde	4 CRC	Injection-site reaction	The vaccine was well tolerated and increased antibody against peptide was noted
Miyagi et al., 2001 (44)	Phase I	SART3 109-118 and SART3 315-323	Montanide	12 advanced CRC	Injection-site reaction	Increased T cell response in those with HLA-A24+ colon cancer cells. The highest dose achieved the best response.
Moulton et al., 2002 (65)	Phase II	β-hCG (CTP37) conjugated to diphtheria toxoid	Mannide monooleate, nor-muramyl dipeptide	77 stage IV CRC	Injection-site reaction	Anti-hCG antibody production was related to better survival with adequate safety issues
Mazzaferro et al., 2003 (66)	-	autologous tumor-derived HSPPC-96	-	29 CRC with liver metastases	No serious adverse events	Significant cytotoxic T cell response was noted. Improvement in OS and DFS in patients with anti-tumor response
Tsuruma et al., 2004 (67)	Phase I	survivin-2B80-88	-	17 HLA-A*2402 positive CRC	No serious adverse events	Survivin-2B80-88 was safe however peptide alone did not elicit overt clinical response
Sato et al., 2004 (45)	Phase I	SART, CyB, Ick, ART	Montanide	10 advanced CRC with HLA-A24+	Injection-site reaction	The combination of these four peptide vaccine was safe. Antigen specific immunity and clinical response was noted.
Mukherjee et al., 2007 (68)	Preclinical	Two MHC I-restricted MUC1 peptides, and, one MHC II helper peptide, mouse unmethylated CpG oligodeoxynucleotide constructs, GM-CSF	IFA	MC38 colon cancer cell line expressing full-length human MUC1 into human MUC1.Tg mice	-	Immunization resulted in robust anti-tumor response. cytotoxic and IFN-γ producing CD4+ lymphocytes against MUC1
Tan et al., 2007 (69)	Preclinical	Porcine and murine endoglin proteins pEDG and mEDG	-	CT26 colon carcinoma mice model	-	DNA vaccine along with mentioned peptides yielded a therapeutic effect better than using them alone
Seledtsov et	Phase I-	xenogenic polyantigenic	-	37 CRC stage	Injection-site	Xenogenic tumor associated antigens were safe

al., 2007 (70)	II	vaccine (B16 and LLC cells, as well as B6 SCs)		IV	reaction	and has the ability to induce cellular and humoral response
Kaumaya et al., 2009 (71)	Phase I	B-cell epitopes of HER2 extracellular domain, promiscuous T-cell epitope	Nor-muramyl-dipeptide, Montanide	24 metastatic and/or recurrent solid tumors	No serious adverse events	Successfully elicited antibody response in more than half of patients
Okuno et al., 2014 (72)	Phase Ib	RNF43, TOMM34, FOXM1 MELK, HJURP, VEGFR1, VEGFR2	UFT/LV, ISA-51VG, Montanide	30 nonresectable mCRC with HLAA*2402-positive	Injection-site reaction	Patients who showed cytotoxic T cell response has longer survival
Kibe et al., 2014 (43)	Phase II	CypB, EGFR, EZH2, NRPL, p56Lck, ppMAPkkk, MRP3, PAP, PSA, PSMA, PTHrP, SART2, SART3, UBE2V, WHSC2	Montanide	60 advanced CRC	Injection-site reaction, anemia, lymphopenia	Increased peptide specific cytotoxic T cells were related to favorable OS
Rahma et al., 2014 (60)	-	13-mer peptides (residues 5-17) corresponding to the tumor Ras mutations	IL-2, GM-CSF, Detox	38 advanced CRC with different ras mutations	Fever, fatigue, injection-site reaction	Peptides and adjuvants successfully induced specific immune response with proposing that adjuvant may have negative effect on immune response
Murahashi et al., 2016 (73)	Phase I	KOC1, DEPDC1, MPHOSPH1, TTK, URLC10 with escalating cyclophosphamide	IL-2 Montanide, ISA-51VG	9 advanced CRC with HLA-A2402-positive	Injection-site reaction	TAA-specific T lymphocyte responses was related to longer survival. Furthermore, higher reduction in regulatory T lymphocytes were correlated with better survival
Correale et al., 2016 (74)	Phase Ib	poly-epitope peptide vaccination to thymidylate synthase	immune-adjuvant IG-1, GOLFIG chemo-immunotherapy	29 mCRC	Injection-site reaction, anemia	Peptide vaccine alone or in combination with adjuvant and chemotherapy showed anti-tumor activity. The combination group showed better survival although adverse event was noted
Goodwin et al., 2017 (75)	Preclinical	Colon cancer peptide antigen (p-AH1-A5)	CpG, 2′3′cGAMP, 5′pppdsRNA, LCP nanoparticle	Female BALB/c mice	-	All 3 adjuvant resulted in proinflammatory response but only 5′pppdsRNA was able to achieve an anti-cancer response
Taniguchi et al., 2017 (76)	Phase I	OCV-C02 consists of two peptides, RNF43-721 and TOMM34-299	Montanide	24 advanced or relapsed CRC with HLA-	Injection-site reaction	OCV-C02 was safe and well tolerated with no dose-limiting toxicity

				A*24:02 positive and no chemotherapeutic response		
Kawamura et al., 2018 (77)	Phase II	HLA-A*2402-restricted RNF43 and TOMM34	UFT/LV, ISA-51, Montanide	44 stage III CRC	Injection-site reaction	Vaccine induced immune response and provided survival benefits

CRC; colorectal cancer; mCRC: metastatic colorectal cancer; OS: overall survival; DFS: disease-free survival; hCG: human chorionic gonadotropin; TSPP: poly-epitope peptide vaccination to thymidylate synthase; MTD: maximal tolerated dose; IFN: interferon; HLA: Human leukocyte antigen; LCP: lipid calcium phosphate; IFA: incomplete freund's adjuvant

Abbreviations:

APC: antigen presenting cells

CEA: carcinoembryonic antigen

CRC: colorectal cancer

CTLA-4 Ab: cytotoxic T-lymphocyte- associated protein-4 antibody

DC: dendritic cells

DCVs: Dendritic cell vaccines

DFS: disease-free survival

GM-CSF: granulocyte-macrophage colony-stimulating factor

hCG: human chorionic gonadotropin

HLA: Human leukocyte antigen

IFA: imiquimod Freund's adjuvant

IFA: incomplete freund's adjuvant

IFN: interferon

IFN: interferon

LCP: lipid calcium phosphate

mCRC: metastatic colorectal cancer

MPLA: monophospholipid A

MTD: maximal tolerated dose

OS: overall survival

PD-1 Ab: death protein-1 antibody

PPV: personalized peptide vaccine

STING: stimulator of interferon genes

TAA: tumor associated antigens

TLR: toll-like receptors

TSPP: poly-epitope peptide vaccination to thymidylate synthase

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