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INS AND OUTS OF CLINICAL TRIALS WITH PEPTIDE-BASED VACCINES

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1. ABSTRACT

Peptides are the smallest antigenic components that are recognized by T cells when presented in MHC molecules on the cell surface. After the identification of peptides from tumor associated and tumor specific antigens, the exploration of the use of peptides in immunotherapy of cancer was instigated. From initial exploration of peptide-mediated induction of immune responses in mice, the peptide based vaccines have evolved to clinical testing in cancer patients. Many different clinical trials have been performed to address the ability of peptide-based vaccines to induce both clinical and immunological responses in patients. This review will provide an overview of the results of the majority of the clinical trials with peptide-based vaccines directed against various antigens in patients with solid tumors.

2. INTRODUCTION

Over one million Americans are diagnosed with cancer every year (1). With a current five-year survival rate of about 60 percent, the cancer mortality rate continues to decline due to refined surgical techniques, improved and combination chemotherapeutic agents, and less invasive radiation procedures. However, the standard therapies often damage healthy cells and tissues, lead to drug toxicities of the kidney and liver, and induce side effects such as nausea and vomiting (70-80%), allopecia, and increased susceptibility to additional tumors (2). These detrimental side effects, as well as the persistent mortality of over 1,500 cancer patients per day in the US, mandates the exploration of alternative approaches to the management of cancer (1). One treatment modality currently being studied is the administration of peptide-based vaccines, a form of immunotherapy aimed at activating the patient's immune system to recognize and defeat malignant tumors (3).

Like all other cells in the body, tumor cells are constantly presenting peptides on their cell surface in the context of major histocompatibility complex (MHC) molecules. Protein antigens from intracellular and extracellular sources are processed into peptides by two different pathways (4). Antigens generated in the cytosol are processed by a multi-subunit proteasome, and the resulting peptide sequences are transported through the ER where they bind to MHC class I molecules that are then transported to the cell surface. These 8-10 amino acid peptides are presented to $CD8^+$ T cells, which are specialized to recognize non-self peptides presented by aberrant cells. Such aberrant cells include virus infected cells and tumor cells, which can be killed by the $CD8^+$ T cells, often referred to as cytotoxic T lymphocytes (CTLs).

Extracellular material that has been taken up by endocytosis is degraded into peptides in endocytic vesicles, and these 12-15 amino acid peptides are bound to MHC Class II molecules within the vesicular system (4). The

resulting complex is transported to the cell surface and can be recognized by $CD4^+$ T helper cells. These helper cells provide crucial assistance in the induction of a CTL response and are also specialized to fight extracellular sources of infection by mobilizing other cells of the immune system such as B cells and macrophages. MHC Class II molecules are expressed by a few specialized cell types of the immune system, which capture and process extracellular antigens. Recognition of the MHC class II-peptide complexes by helper T cells activates the T cell through the T cell receptor-CD3 complex and the antigen presenting cell through CD40-CD40 ligand interaction (5). The T helper cell will then produce cytokines that will enable the CTL to be activated by the same antigen presenting cell. This process takes place in the lymphoid tissue and the CTLs will subsequently migrate to the tumor to perform their cytolytic task. Activation of the immune system against the tumor may occur naturally in tumor bearing patients; however when not present, the immune response can be induced through active vaccination.

Justification for the use of immunotherapy in the treatment of cancer is found in several instances where treatment by immune activation has been successful. In addition, increased incidence of certain types of malignancies has been observed in immunocompromised patients (6). The rationale behind tumor immunotherapy is to identify antigenic protein targets in the patient's tumor and present these proteins to the patient in such a way that an immune response against the tumor is initiated. One of these vaccination methods employs synthetic MHC class I or MHC class II restricted peptides from tumor specific or tumor associated antigens. This review will provide an overview of the results obtained in clinical trials of peptide-based vaccines against a number of solid tumor targets in cancer patients. These results are also reflected in a summary table. Overall conclusions and perspectives will be presented in the final chapter.

3. HER-2/NEU

HER-2/neu is a member of the epidermal growth factor receptor family and functions as a growth factor receptor. This transmembrane protein consists of an extracellular domain (ECD), which binds ligand and an intracellular cytoplasmic domain (ICD), which is involved in signaling (7). The HER-2/neu gene is present in normal cells as a single copy. Amplification of the gene and/or overexpression of the associated protein have been identified in many human cancers including breast, ovary, and adenocarcinoma of the lung (8). Peptides of this protein that bind to MHC class I and MHC class II have been identified. Initial clinical vaccination strategies have concentrated on eliciting a $CD4^+$ T helper response. A vigorous T helper response may serve to augment the production of HER-2/neu antibodies and/or HER-2/neu specific CTLs, both of which could be therapeutic.

Two clinical trials have been completed thus far examining both the safety of HER-2/neu peptide-based vaccines and whether detectable immunity to the HER-2/neu protein can be generated. The initial study focused on eliciting HER-2/neu specific $CD4^+$ T helper responses (9). Patients were randomized to receive one of two helper T cell vaccines, one that targeted the ICD, and one that targeted the ECD, each composed of three peptides, 15-18 amino acids in length. GM-CSF, which induces dendritic cells locally, was used as an adjuvant. The immunization was given intradermally monthly for six months. The patients in the study had received previous treatment for stage III/IV breast, ovarian or lung carcinomas that overexpressed HER-2/neu in the primary tumor or metastases and were without evidence of disease or in a minimal residual disease state at the time of enrollment. Initial analysis was completed following entry of the first eight patients into the trial. Four of eight patients completed the trial. Of these four, none had HER-2/neu specific $CD4^+$ T cell response before vaccination and all developed peptide specific $CD4^+$ T cell responses after at least two vaccination cycles. Six of the eight patients developed HER-2/neu protein specific $CD4^+$ T cell responses as well. Epitope spreading, which indicates the generation of immune response to portions of protein not included in the vaccine, was also observed in a majority of patients. This suggested that HER-2/neu was being processed and presented naturally by the tumor in an augmented fashion. Delayed type hypersensitivity (DTH) responses developed at the injection site after multiple vaccinations in all patients and at distant sites in two of the patients who completed all six injections. No toxicity was noted against tissues that expressed basal levels of HER-2/neu including skin, GI tract epithelium and lung.

After 32 patients had completed their course of six HER-2/neu peptide vaccinations, the data were examined in a separate publication to correlate patient's peptide specific DTH response at a site distant from the vaccine site to their peripheral blood specific T cell response (10). Patients were skin tested on the back one month after their last vaccination. HER-2/neu peptide specific T cell responses were measured at baseline and 30 days after each vaccination. DTH was categorized as >10 mm^2, between 5-9 mm^2, <5 mm^2. HER-2/neu peptide peripheral blood T cell responses were defined by stimulation index (SI) as SI >2.0 or SI<2.0. It was concluded that the odds of having an SI>2.0 increased as DTH response increased. DTH responses >10 mm^2 correlated significantly with a measurable antigen specific T cell response.

A more recent clinical trial sought to determine whether HER-2/neu specific CD8+ T cell immunity could be elicited using a HER-2/neu derived MHC class II helper peptide, which contained an HLA-A2 class I binding motif within its sequence (11). The trial tested a T helper vaccine composed of three peptides, 15-18 amino acids in length, including one that targeted the ICD, one that targeted the ECD and one that included a helper peptide that contained an HLA-A2 binding peptide within its sequence. GM-CSF was used as an adjuvant. Nineteen HLA-A2 patients who had previous treatment for stage III/IV breast, ovarian or lung carcinomas that overexpressed HER-2/neu in primary tumor or metastases and were without evidence of disease or in a minimal disease state at the time of enrollment were included in the study. HER-2/neu specific $CD4^+$ T cell

proliferation responses were measured at baseline, before each vaccination and at the end of the study. Ten day ELISPOT assays were used to determine precursor frequencies of peptide specific $CD8^+$ T lymphocytes. Data was reported on 18 patients who received at least one vaccine, 14 of whom completed all six vaccines. Fourteen of 18 patients had proliferative responses to at least one of the HER-2/neu peptides contained in their vaccine formulations (11). Patients immunized with peptides also developed responses to the naturally processed HER-2/neu protein. Proliferative responses to the ECD were detected in 5/18 patients compared to 0/19 at baseline and to the ICD in 9/18 patients compared to 1/19 at baseline. Immunization with 15 amino acid HER-2/neu peptides increased CD8+ T cell precursors specific for HLA-A2 restricted 9 amino acid peptide epitopes contained within their sequence. After immunization, overall CTL responses were increased. According to chromium release assays, peptide specific T cells were able to lyse HLA-A2 EBV transformed lymphoblastoid cells expressing HER-2/neu protein in 25% of cases. Five patients followed for 7-17 months after the final vaccination maintained $CD8^+$ T cell responses to two or more peptides in the vaccine (11).

4. HUMAN PAPILLOMAVIRUS

Integration of the high-risk human papillomaviruses (HPV) type 16 and 18 into the genome leads to overexpression of the E6 and E7 proteins of HPV16 and HPV18. The E7 protein binds to the hypophosphorylated form of Rb and displaces E2F transcription factors(12) and the E6 protein binds to and facilitates the degradation of p53 (13). Thus both E6 and E7 disable tumor suppressor proteins and lead to the development of cancer (14). High risk HPV has been identified as a causative agent for cervical cancer, but an increasing number of reports have also detected HPV DNA in other tumors like penile, anal and pharyngeal carcinomas (15). Immunogenic peptides of different HPVs have been identified for many HLA haplotypes and papillomavirus proteins (16). This allowed the design of vaccines to enhance immunoreactivity to epitopes of HPV. Three clinical trials have been completed thus far examining responses of cervical cancer to peptide-based vaccines. $CD8^+$ T cell response to class I epitopes derived from E6 and E7 proteins was the primary measure of immunoreactivity in each of these studies.

The first clinical trial tested the ability of multiple vaccinations with a lipidated HLA-A2*0201 restricted HPV16 E7 aa 86-93 peptide linked to PADRE, a non specific helper epitope, to elicit cellular immune responses (17). Twelve women expressing HLA-A*0201 with refractory cervical or vaginal cancer received four inoculations of E7-PADRE lipopeptide at three week intervals (18). Induction of epitope specific $CD8^+$ T cell response was measured by IFN-gamma release assay. Clinical response was determined by physical exam and radiological evaluation. Results showed no clinical responses or treatment toxicities other than skin discomfort at injection site. Six patients were unable to complete the full course due to rapidly progressing disease. After all four vaccines, two patients were alive with stable disease and three patients showed progressive disease.

A second clinical trial was designed as a dose escalation study in which successive groups of patients received 100, 300, or 1000 µg of two HPV E7 peptides (aa 11-20 and aa 86-93) and the PADRE helper peptide, respectively, emulsified in Montanide ISA 51 adjuvant (19). Nineteen HLA-A*0201 positive women with HPV16 positive cervical cancer were given a total of four vaccinations, one every three weeks. Eleven of 19 patients demonstrated a low absolute lymphocyte count pre- and post vaccination indicating an immunocompromised state, possibly due to previous chemotherapy or radiation. Two patients who had normal lymphocyte count prior to vaccination demonstrated a low count after vaccination. No other adverse effects were seen except for temporary erythema in four patients and local indurations in two patients, which subsided in six to eight weeks. Biopsies taken at vaccination sites showed infiltration of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, which may have been a reaction to the vaccine. Two patients maintained stable disease for over one year after vaccination, but all other patients experienced progressive disease. Lymphocytes were sampled before the first and after the last vaccination. Immunological analysis revealed no induction of CTL responses against HPV16 E7 peptides at any dose (20). Despite three month washout after other therapies, all patients showed reduction in influenza specific CTLs. On the contrary, after vaccination, strong PADRE helper peptide specific proliferation was detected in four of 12 patients, indicating that some immunocompromised patients can demonstrate $CD4^+$ T cell specific immunity (20).

In the most recent clinical trial, 18 HLA-A2 positive patients with colposcopy and biopsy proven Grade II/III HPV16 positive cervical or vulvar intraepithelial neoplasia received escalating doses of four immunizations at three week intervals (21). The first 10 patients received one nine aa peptide, aa 12-20, from HPV16 E7 emulsified with incomplete Freund's adjuvant. Starting with the 11^{th} patient, an eight amino acid peptide, aa 86-93, linked to a lipidated helper T cell peptide with a covalently linked lipid tail was added to the 2000 mcg dose of the original peptide. Seventeen of 18 patients showed minor toxicity to the vaccination developing granuloma formation with erythema, edema and warmth persisting for three weeks. Grade II lethargy, weakness, nausea, diarrhea and fever occurred in patients on all doses. Repeat colposcopy and definitive removal of dysplastic tissue completed three weeks after the fourth immunization revealed that 9 of 17 patients had at least partial regression of their lesions with three patients having complete regression. There was no T cell change, but there was an increase in dendritic cell infiltrate in the dysplastic lesion of all six patients tested. Cytokine release and cytolysis assays on the peripheral blood mononuclear cells (PBMC) showed an increase in E7 specific CTL reactivity in 10 of the 16 patients. No positive DTH was found in any patient before or after vaccination. Twelve of 18 patients had PCR confirmed viral clearance from cervical scraping by the fourth injection but all biopsy

samples were still PCR positive for HPV16 at the RNA level (21).

5. PSMA

All peptide vaccines tested thus far in patients with prostate cancer have been based on two HLA-A*0201 restricted peptides of the prostate-specific membrane antigen (PSMA). Prostate epithelial cells express PSMA and overexpression has been detected in hormone refractory prostate carcinomas (22). Elevated levels of PSMA have been observed in the serum of patients with advanced prostate cancer (23). The clinical trials performed with the two PSMA peptides have all used autologous dendritic cells (DCs) to deliver the peptides to the patients. DCs, being the ultimate antigen presenting cells, express all accessory molecules that are required to induce an optimal CTL response. Loading CTL peptides on DCs is regarded as a good alternative to oil based adjuvants, like Freunds or Montanide ISA 51. For all trials with PSMA loaded DCs, the DCs were obtained from peripheral blood mononuclear cells that were cultured in 5% autologous plasma in the presence of 1000 units GM-CSF and 1000 U IL-4.

A phase I study enrolled 51 patients with advanced metastatic prostate cancer in five different treatment arms: 1) peptide 1; 2) peptide 2; 3) autologous DC; 4) DC+peptide 1 and 5) DC+peptide 2 (24). Patients received four or five infusions over six to eight months (six week intervals). No significant acute or chronic toxicity was observed in any group as a result of the treatment. Seven partial responders as determined by >50% reduction of PSA levels were reported, five of whom were in the groups that received peptide pulsed DCs. The clinical response in four of the seven responders lasted over 200 days. Remarkably, two of these four patients were HLA-A2 negative and therefore, the response was not induced by the peptides delivered through the vaccination (25). Only one of the HLA-A2 positive responders received DCs+peptide, the other received just peptide 2. CTL responses against peptide 1 or 2 were identified in groups 4 and 5 but did not necessarily coincide with the clinical responses.

A phase II trial enrolled 107 patients with hormone refractory metastatic prostate cancer, who received six infusions with peptide 1 and 2 loaded autologous DCs (average 17 million per injection) at six week intervals (26). Only 62 patients, 25 of whom also participated in the phase I trial, were evaluable; 30% responded to the therapy with a median clinical response of 160 days. Responses were based on criteria of the National Prostate Cancer Project, plus 50% reduction of PSA levels or resolution of a previously measurable lesion on a ProstaScint® scan. Of the 19 clinical responders, three patients had a complete response but one of these three was HLA-A2 negative (27). The immunological evaluation of this trial indicated no correlation between clinical responses and immunological responses. Both delayed type hypersensitivity (DTH) tests for recall antigens and interferon-γ production by PBMC after anti-CD3 stimulation showed no difference when evaluated before and after treatment (28). Some immune responses upon vaccination were reported against the PSMA peptides, but the overall conclusion was that there was no clear correlation between clinical responses upon treatment with DCs loaded with PSMA peptides and peptide specific immune responses (29).

A phase II trial in 37 patients with recurrent prostate cancer showed similar results as previous trials. A 30% clinical response rate, including two of 11 responders who were HLA-A2 negative, was reported (30). Increasing the number of DCs per injection and decreasing the number of injections did not significantly change the outcome of therapy as determined in another trial with 28 patients (31).

6. CEA

The carcinoembryonic antigen (CEA) family consists of 29 genes located on chromosome 19 and has been identified as a member of the immunoglobulin superfamily. CEA is considered an adhesion molecule and its overexpression on the majority of colorectal, gastric, pancreatic, breast and non-small-cell lung carcinomas makes CEA an attractive target for immunotherapeutic treatment of these malignancies (32). Several different forms of immunotherapy have been directed towards CEA, including monoclonal antibody therapies, DNA based vaccination with or without different viral delivery vehicles and peptide vaccination (33). All three phase I clinical trails with CEA peptides have used autologous dendritic cells to deliver the same HLA-A2 restricted CEA derived CAP-1 peptide. The first trial enrolled 21 patients with CEA expressing metastatic malignancies originating from colon, breast, ovary, pancreas and ampulla of Vater. DCs were delivered at three different doses and all patients, except for one, had four doses. Clinical evaluation of 19 patients revealed only one patient with a minor response and one with stable disease. The immunological evaluation showed no increased cellular immune reactivity after peptide vaccinations as determined by DTH responses (34).

In another study done by the same group, the CTL responses against the CAP-1 CEA peptide and a Flu peptide were evaluated in an ELISPOT assay after three monthly vaccinations with CEA peptide in Detox/PC adjuvant (35). Two groups of 12 patients received either 1 or 3 mg doses subcutaneously (s.c). at two different sites. The patients seemed to have a reduced CTL response against the Flu peptide prior to vaccination indicating a general immunosuppressed state in the cancer patients as compared to the healthy donors. Six of 16 patients showed a CEA response after peptide vaccination, but most of them also had a CTL response before vaccination, and responses were therefore not considered a result of the treatment.

A third trial in seven patients studied the treatment with CAP-1 peptide and calcitonin loaded DC against medullary thyroid carcinoma (36). The therapy was found to have no serious adverse effects and clinical responses included one patient with partial response, one with mixed response, three with stable diseases and two with progressive disease. Immunologically, all patients developed a DTH response against the peptide pulsed DC;

however, earlier studies had revealed that these responses are not necessarily directed against the peptide but could be against the DCs.

7. MUC1

Mucin-1 (MUC1), a member of the mucin family of transmembrane glycoproteins, is composed of a polypeptide core containing multiple tandem repeats of a 20 amino acid sequence (referred to as VNTR) which is highly glycosylated by O-linked carbohydrate side chains (37). Mucins are produced by cells of epithelial origin and are abundant on the apical surface of these cells as they form glands. In cancer, MUC1 is overexpressed and often present over the entire cell surface instead of being restricted to the luminal surface as in normal tissues. In addition, abnormal MUC1 glycosylation in cancer cells results in less complex and fewer carbohydrate side chains (37). Therefore, in tumors, there is increased exposure of MUC1 epitopes to the immune system. These epitopes can induce both an antibody and cytotoxic T lymphocyte response, both of which are being exploited in phase I clinical trials of MUC 1 peptide-based vaccines (38).

The first phase I clinical trial used MUC1 synthetic peptides to determine the toxicity and immunogenicity of MUC1 core peptides (39). Thirteen patients with established breast cancer and metastatic disease were injected with increasing doses (150-1000 mcg) of the peptide Cp 13-32 (CPAHGVTSAPDTRPAPGSTAP) of the MUC1 VNTR conjugated to diphtheria toxin. No toxicity was found other than a DTH to the diptheria toxin. Weak antibody responses, T cell proliferative responses and DTH reactions against MUC1 were demonstrated in a proportion of patients.

In another study, MUC1 synthetic peptide containing 105 amino acids (5 VNTR repeats) was used together with BCG to inject 63 patients with proven adenocarcinoma of the pancreas, breast or colon at 0, 3, and 6 weeks (40). All patients were able to tolerate therapy with only local ulceration at the vaccination site. Only three of the patients demonstrated strong skin response to DTH injection of the MUC 1 peptide, but 37 of 55 biopsies showed intense T cell infiltration at the site of the 105 amino acid peptide injection. Stored PBMCs were examined and seven of 22 patients had a two to fourfold increase in mucin specific CTL precursors after vaccination. Forty-three patients experienced symptoms that worsened as the trial progressed such as fever, sweats and malaise. Serum level of IL-6 correlated with presence of these symptoms. While none of the patients had partial or complete response according to MRI and CT, three had stabilization of disease.

In a separate study, 25 patients with metastatic adenocarcinoma of the breast, colon, rectum and stomach were immunized with oxidized MUC1 fusion protein consisting of 5 VNTR repeats (PDTRPAPGSTAPPAHGVTSA) linked to conventional carriers (41). The vaccine was chemically conjugated to mannan, a delivery system which targets the MUC1 peptides to the class I pathway for their subsequent presentation to $CD8^+$ T cells. Injections were given s.c. at wks 1,2,3,4,7,9,11,13 with doses ranging from 10-500 mcg. Nine patients received four injections and 16 received the full course of eight injections. Results showed low toxicity. After vaccination, 13 of 25 injected patients were positive for MUC1 antibodies with a positive correlation between antibody levels and the dose of MUC1 fusion protein received. CTL reactivity against MUC 1 epitopes (STAPPAHG or PAPGSTAP) could be identified in seven patients. Induction of IgG anti MUC1 antibody suggested involvement of T cell help. A proliferative cellular response to MUC1 in four of 14 patients indicated that MUC 1 could stimulate $CD4^+$ T cells in vivo. Clinical outcome of this study has not been reported.

A fourth phase I clinical trial describes the generation of MUC1 class I restricted CTLs in 16 patients with recurrent metastatic breast cancer (42). Patients were immunized with a low dose (5 µg) of a 16 amino acid MUC1 peptide (GVTSAPDTRPAPGSTA) conjugated to keyhole limpet hemocyanin (KLH), an immunogenic protein carrier, plus DETOX adjuvant on weeks 0,2,5, and 9. While all patients generated a strong anti-KLH IgG response, only three patients developed anti MUC1 IgG responses. However, according to chromium release data after a six day in vitro peptide stimulation, seven of 11 patients produced class I restricted anti-MUC1 CTLs. From these data it is not clear whether these CTLs were a result of the vaccination.

In another phase I clinical trial, nine patients with history of breast cancer without evidence of current disease received a MUC1 peptide vaccine containing 100 mcg of 1.5 repeats of the 20 amino acid MUC1 sequence (C-VTSAPDTRPAPGSTAPPAHGVTSAPSTRPA)
conjugated with KLH and premixed with 100 µg immune adjuvant QS-21 (43). The s.c. injections were administered at weeks 1,2,3,7,19. Local skin reaction at the site of vaccination of four to five days duration and mild flu like symptoms of one to two days duration were noted. Toxicities also included inguinal adenopathy near injection site (two patients), transient leukopenia (two patients) and grade 2 neutropenia (two patients). A long lasting (106-137 weeks) significant increase in IgM and IgG antibody titers against MUC1 were detected in all patients (43). Binding of IgM to MUC1 expressing MCF-7 tumor cells was observed in seven patients. There was no evidence of T cell activation. Two patients developed recurrence of disease but all others were without recurrence after a median of 135 weeks. Antibodies were found to bind primarily to the APDTRPA epitope of MUC1 (44).

The most recent trial reported three phase I studies of a MUC1 antigen that consisted of an 100 amino acid construct containing five VNTR repeats produced as a GST fusion protein (45). The GST-MUC-1 protein was chemically fused to mannan, in order to target the protein to the mannose receptor for cellular uptake, as previously tested (41). In this study, cyclophosphamide and intraperitoneal (i.p.) route of administration were assessed

to examine their effect on immune response. Forty-one patients with metastatic or locally advanced breast cancer, colon cancer, and various adenocarcinomas received increasing doses (1-300 mcg) of MUC1 fusion protein at weekly intervals for three weeks and then at weeks 7 and 9. Cyclophosphamide was given on weeks 1 and 4 in an attempt to increase cellular immunity. MUC 1 fusion protein was given intramuscularly (i.m.) to the breast cancer patients, i.m. to adenocarcinoma patients and i.p. to the colon cancer patients in three separate trials. A symptom of toxicity was erythema at the injection site and only microscopic DTH responses were seen in response to intradermal injections with VNTR. Sixty percent of patients had a high titer of MUC 1 IgG with i.p. injections, yielding a 10 fold higher response than other vaccination routes. This increased antibody responses was attributed to the cyclophosphamide. Cellular responses were found in 28% of patients. Cyclophosphamide was of no significant benefit in eliciting a CTL response. Only five of the 41 patients showed stable disease while all other patients progressed (45).

8. RAS

The proto-oncogenes of p21 *ras* (K-*ras*, H-*ras* and N-*ras*) are mutated in a wide variety of tumors including carcinomas of the colon, lung, pancreas and melanomas (46). The most sensitive codons for point mutations are codon 12 and 61. The normal glycine at codon 12 is most frequently changed into an aspartic acid (Asp), valine (Val), or cysteine (Cys). Mutations in *ras* result in the production of an altered p21 G protein that is continuously active and may lead to transformation and tumorigenesis. The point mutations in *ras* could lead to the formation of new epitopes that upon binding in the HLA molecules would induce a mutant ras specific T cell response. Such mutant Ras specific T cells should recognize tumor cells that express the mutant Ras. Several murine and human immunogenic epitopes for MHC class I and II have been identified for K-ras, mutated at codon 12, 13 and 61 that can induce both $CD8^+$ and $CD4^+$ T cell responses (47).

The first clinical trial targeted mutant K-ras in pancreatic carcinoma patients by administering multiple intravenous injections of mononuclear cells loaded with a ras peptide aa 5-21 containing a mutated amino acid at position 12 that corresponded with the ras mutation in the patients' tumors (48). Two of five patients displayed proliferative T cell responses after completion of the vaccinations, but the clinical evaluation did not reveal any responses (49). From one patient, two responding $CD4^+$ T cells were cloned and found to be restricted to HLA-DR6 and DQ2. The DR6-restricted T cells were able to kill autologous cells pulsed with the ras peptide containing the Val 12 mutation present in this patient's tumor (50).

A second trial tested vaccination with K-ras peptides aa 5-17 with codon 12 mutations corresponding with the patients' tumors. The 15 patients were divided into five dose groups (100, 500, 1,000, 1,500 and 5,000 mcg) and each received three monthly subcutaneous injections of the peptide in Detox adjuvant. Despite the detection of both $CD4^+$ and $CD8^+$ T cell responses directed against mutant ras peptides after vaccination in three of 10 evaluable patients, no clinical responses were obtained. The CTL responses were found to be HLA-A2 restricted, indicating that the ras mutation 12 Gly to Val gives rise to an HLA-A2 restricted immunogenic epitope (51).

Intradermal vaccination with mutant K-ras peptides in GM-CSF was administered to 48 patients with pancreatic carcinoma in a phase I/II trial. Twenty-five of the 43 evaluable patients showed either specific T cell responses or DTH responses against the ras peptides, and mutant ras specific $CD4^+$ and $CD8^+$ T cells were isolated from the patients after vaccination. In addition to these immune responses, several clinical responses were observed. Overall, patients who had an immune response against the peptide vaccine had a significantly longer median survival than the patients that did not show immune responses (52).

Recently, a trial was performed with N-ras derived peptides bearing mutations at codon 61. N-ras mutations are frequently observed in melanomas and this clinical trial tested six intradermal injections of mutant N-ras peptides aa 49-73, using GM-CSF as an adjuvant in 10 melanoma patients. Eight of the 10 patients responded with a DTH response against the peptide after vaccination and ras-specific $CD4^+$ T cells were obtained from the peripheral blood of 2 patients. This study shows again that it is possible to activate T cell responses against mutant ras proteins expressed in tumors by vaccination with synthetic peptides (53).

9. MELANOMA

Melanoma has the longest history of all the types of cancer being targeted by immunotherapy. Peptide-based vaccinations have been tried in many melanoma patients, targeting different melanoma associated antigens with various possible delivery methods and adjuvants. As anti-melanoma vaccines are leading the field of cancer immunotherapy, the data from the trials may be indicative of the results that will be obtained from clinical trials targeting other malignancies. A number of recent reviews contain the results from clinical trials on peptide-based therapies against melanoma (54-57) and therefore an additional summary of the results from clinical trials on melanoma will not be provided in this review.

10. CLINICAL TRIALS WITH PEPTIDE-BASED VACCINES SUMMARY TABLE

(Table 1).

11. CONCLUSIONS AND PERSPECTIVES

Many clinical trials using peptide-based vaccines against cancer have been performed and others are currently ongoing. While it may be too early to draw firm conclusions about the efficacy of peptide-based vaccines from these trials, this review of clinical trial results may serve to guide future trials. Since most of the research on

Table 1. Clinical trials with peptide-based vaccines summary

Protein	Peptide Epitope	Cellular Response	Clinical Response	Side Effects	Reference
HER-2/neu	ICD or ECD (each 3 peptides 15-18aa)	CD4+ DTH	Not examined	No toxicity	9,10
HER-2/neu	ICD or ECD and HLA-A2 binding peptide	CD4+ CD8+	Not examined	Skin rash	11
HPV16E7	aa 86-93+PADRE	CD8+	None	Skin discomfort at injection site	17,18
HPV16E7	aa 11-20 + 86-93 + PADRE	CD4+ CD8+	Not examined	Lymphopenia, local erythema	19,20
HPV16E7	aa 11-20/86-93	CD8+	9/17 partial regression 12/18 Cervical scraping HPV (-)	Granuloma,erythema, diarrhea, N/V	21
PSMA	PSM-P1(LLHETDSAV) PSM-P2(ALFDIESKV)	CD8+	7/51 Reduction in PSA	Moderate transient hypotension	24,25
PSMA	PSM-P1 PSM-P2	DTH CD8+	19/62 Reduction in PSA 6/25 Partial response 2/25 Complete response	Not reported	26-29
PSMA	PSM-P1 PSM-P2	DTH CD8+	11/37 Reduction in PSA	Low toxicity	31
CEA	CAP-1(YLSGANLNL)	None	1/19 minor response	Low toxicity	34
CEA	CAP-1	CD8+	Not examined	Not examined	35
CEA	CAP-1	DTH	1/7 partial response	None	36
MUC1	VNTR(Cp13-32)	AntibodyCD8+ DTH	Not reported	None	39
MUC1	VNTR(5 repeats)	DTH CD8+	None	Local ulceration, fever, malaise	40
MUC1	VNTR(5 repeats)+mannan	Antibody CD8+ CD4+	Not reported	Low toxicity	41
MUC1	GVTSAPDTRPAPGSTA +KLH	Antibody CD8+	Not reported	Not reported	42
MUC1	VNTR (1.5 repeats) +KLH	Antibody	Recurrence of disease in only 2/9 high risk patients	Local skin reaction, mild flu symptoms	43
MUC1	VNTR (5 repeats) + GST + mannan	DTH Antibody CD8+	None	Erythema at injection site	45
K-ras	ras (aa 5-21)	CD4+	None	No toxicity	48-50
K-ras	ras (aa 5-17)	CD4+ CD8+	None	No toxicity	51
K-ras	ras (aa 5-21)	CD8+ CD4+ DTH	Increased median survival	No toxicity	52
N-ras	ras (aa 49-73)	DTH CD4+	Not examined	Not examined	53

ICD-Intracellular Domain, ECD Extracellular Domain, DTH-Delayed Hypersensitivity reaction, VNTR(PDTRPAPGSTAPPAHGVSTA)- a 20 amino acid sequence which is highly glycosylated by O-linked carbohydrate side chains

peptide vaccines is in phase I trials, it is important to conclude that none of the peptides themselves or their delivery methods have shown significant acute toxicities. Erythema, weakness, nausea and some mild leukopenia have been reported, but the overall data show that the delivery of peptides in various adjuvants is safe and well tolerated. The clinical response rates in patients vary greatly depending on the study, but tend to be low. Several factors may have contributed to this low response rate, including previous chemo-and/or radiation therapies, the advanced tumor stage of most participants and the immune escape capacities of the tumors. The latter may explain the observation that the immune responses are generally more profound than the clinical responses. In contrast, one study convincingly showed a significant positive correlation between the induction of an immune response by the

vaccine and median survival (52). No preferred adjuvant or delivery route has been identified as of yet, and no studies have been designed to pursue this. Some peptides induced better responses than others, but this may have been related to the adjuvant rather than the peptide. Therefore, future studies should be designed to evaluate the responses to the same peptide in different adjuvants.

Most immunotherapeutic approaches are expected to be more effective in settings where tumor masses are small and immune responses are good. However, patients enrolled in most of the phase I clinical trials have advanced disease and a sub optimal immune system as a result of prior therapies and the immunosuppressive effects of growing tumors. Therefore, patients with minimal residual disease or early precursor lesions like cervical or prostate intraepithelial neoplasia (CIN and PIN) would be more suitable for peptide-based vaccines, and clinical results could be more substantial.

There is good evidence that an immune response against a single peptide will not be curative, as this will favor antigen down regulation or outgrowth of antigen negative tumor cells. (58). Therefore, future vaccines may benefit from including multiple epitopes from different proteins, as these are more likely to overcome immune escape mechanisms and result in a curative immune response. The induction of a memory CTL response is regarded as an important result of the vaccination and the inclusion of helper peptide epitopes in peptide-based vaccines will improve the memory responses. In some of the trials discussed above, the $CD4^+$ helper T cells not only facilitated CTL and memory T cell induction, but also exerted cytolytic activity by themselves (50).

The improvements and refinements of peptide prediction programs (http://bimas.dcrt.nih.gov/molbio/hla_bind/, http://www.imtech.res.in/raghava/propred/), and the proteasome cleavage algorithms available (59, 60), (http://www.paproc.de/expl2.html, http://www.cbs.dtu.dk/services/NetChop/), will increase the speed and accuracy of peptide identification from new and established tumor antigens for different class I and class II HLA haplotypes. This will facilitate an increase in the number of clinical trials with peptide-based approaches. The trials perfumed to date show hopeful immunologic results, and improvements like new adjuvants, inclusion of helper epitopes and the opportunity to select immunocompetent patients with early tumors or minimal residual disease, are likely to increase the clinical efficacy of peptide-based vaccines during the next decade.

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