Current Status Of Short Synthetic Peptides As Vaccines

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Abstract: Preventative medicine in the form of vaccination had a huge impact on human health in the 20th Century. Vaccines are now recognized as the most effective line of defence against infectious agents that cause disease and death, and in some cases vaccines have enabled complete eradication of disease from the globe (e.g. smallpox). Nevertheless there are still many human diseases (e.g. viral and parasitic infections, cancers) for which there are no effective vaccines. Current vaccines are mainly live and attenuated viruses or viral, bacterial or recombinant proteins and polypeptides. By virtue of their natural aminoacid composition, polypeptides and proteins are relatively safe materials for vaccination, but they are expensive to manufacture making them inaccessible to the most vulnerable and needy human populations that cannot afford such medicines. This review will focus on shorter synthetic peptides that are cheaper to manufacture, conceivably even safer for human use because of increased specificity, but they also suffer from problems that have presumably resulted in their lack of progress in clinical trials. Since 1990, over 100 chemically synthesized short peptide vaccines have entered Phase I clinical trials, less than 20 have progressed into Phase II, but none have entered Phase III clinical trials. In this review we discuss reasons why vaccines based on short peptides may not have succeeded in the clinic, identify problems such as insufficient immunogenicity, structural/conformational instability, chemical instability due to degradation, and describe possible solutions to some of these problems that have been investigated in recent years.

Key Words: Peptide, vaccine, adjuvant, clinic, epitope.

INTRODUCTION TO VACCINES

The development and preventative use of vaccines during the 20th Century resulted in many spectacular successes in protecting against, and in some cases nearly eliminating, infectious diseases that had so devastated human populations in previous centuries. Historically, the principle of vaccination developed through inoculation with a dead or modified pathogenic organism to initiate a mild form of the disease it was responsible for, leading to immunity to later natural attacks by that organism. As early as the 17th century it was known that milkmaids, and others who had contracted cowpox, were immune to the deadly smallpox virus and Jenner's seminal experiments reported from 1798 started the scientific path towards rational immunization approaches using vaccination to prevent epidemics of a wide variety of diseases [1].

Early successes against rabies, anthrax, typhoid fever, yellow fever and smallpox, eventually led health authorities in more affluent countries to conduct widespread vaccination programs against a diverse variety of pathogens throughout the 20th century. These proved to be spectacularly successful preventative health measures, but were largely directed against diseases that threatened those affluent populations, particularly children. Today children in many developed countries are routinely vaccinated against hepatitis B, diphtheria, tetanus, pertussis (whooping cough), poliomyelitis, haemophilus influenza type B (Hib), measles, mumps, rubella, chicken pox, pneumococcus, meningicoccus, and tuberculosis, and effective vaccines are also available for many other human diseases (e.g. hepatitis A, Japanese encephalitis, meningitis, pneumonia). The effects of vaccination are rarely permanent and thus eradication of disease from a given sub-population relies upon the vigilance of the population and health authorities in maintaining effective, comprehensive, repetitive and widespread vaccination programs supported by effective public education programs. Surveys of people who do not get their children vaccinated suggest that they may lack sufficient information about the efficacy and safety of particular vaccines and/or the importance of vaccination programs [2].

There are various different types of vaccines available for human use, including DNA-based vaccines, cellular vaccines, live virus/bacterium/parasite vaccines, attenuated or inactivated vaccines, and vaccines based on bacterial proteins, recombinant protein vaccines, and DNA vaccines [3], and some examples are surveyed in Table **1** [4]. Factors that are considered in the choice of vaccine type are the epidemiology of disease, recipient (adult/children or both), the nature and cost of available vaccine (live attenuated or inactivated), genetic variation of the population, and risks of side effects. Today, there is still an urgent need for novel, more effective, more easily deliverd, safer, cheaper vaccines and more effective vaccination methods to prevent life threatening diseases such as cancers, neurodegenerative diseases, viral infections (e.g. HIV/AIDS, influenza, flaviviruses), and parasitic diseases (e.g. malaria, schistosomiasis) that still claim many lives.

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Table 1. Some Vaccines Currently Available for Human Use Against Bacterial/Viral Diseases (Modified from [4])

Vaccine	Infectious agent	Disease
Inactivated	B*acillus anthracis*	Anthrax
	B*acillus pertussis*	Whooping cough
	V. *cholerae*	Cholera
	Hepatitis A	Liver disease, Cancer
	Influenza virus A	Respiratory disease
	Japanese encephalitis virus A	Brain infection
	Polio virus A	Poliomyelitis, paralysis
	Rabies virus	Rabies
	C*oxiella burnetii*	Q fever
Live attenuated	M. *tuberculosis*	Tuberculosis
	V.*cholerae*	Cholera
	Adenoviruses	Respiratory disease
	Hepatitis A	Liver disease
	Influenza virus A	Respiratory disease
	Measles virus	Respiratory tract infection, encephalitis
	Mumps virus	Mumps, meningitis, orchitis
	Poliovirus	Poliomyelitis, paralysis
	Rubella virus	German measles, fetal malformations
	Vaccinia virus	Smallpox
	Varicella-zoster virus	Chickenpox
	Yellow fever virus	Jaundice, kidney and liver failure
	Herpes zoster virus	Shingles
	Rota virus	Gastroenteritis
Subunit	Hepatitis B	Liver disease, cancer
	B*accilus pertusis*	Whooping cough
	B*orrelia burgdorferi*	Lyme disease
Toxoid	C*lostridium tetani*	Tetanus
	C*ornybacterium diphtheriae*	Diphtheria
Cojugated	H. *influenzae*	Meningitis, epiglottitis, pneumonia type b
	N*eissria meningitidis*- C	Meningitis
	S. *Pneumoniae*	Pneumonia, otitis media, meningitis
Recombinant	Human Papillomavirus	Cervical cancer

PEPTIDES AS VACCINES

There are two kinds of peptide vaccines, recombinant polypeptides/proteins and synthetic peptides. This review will focus mainly on short synthetic peptides which have been investigated as vaccine candidates for over 40 years. For a peptide to be a vaccine, it must be immunogenic and this is usually the first barrier that needs to be overcome because short peptides do not generally induce a strong immune response. A major advance in peptide vaccine research was the finding of certain carrier proteins and adjuvants that could amplify the immune response (*vide infra*), as measured *in vitro* by ELISA assays for peptide-antibody binding [5]. The discovery of immunogenicity for polypeptides, and for some short peptides in the presence of an adjuvant or carrier molecule, ushered in a promising new era in potential therapeutics based on vaccination [6, 7]. In reality however, short peptide vaccines have still not yet realized their initial promise, even though they have a number of advantages over more conventional vaccination approaches.

Immunological properties of peptides depend largely on the properties of the host being immunized (e.g. immunoglobulin gene repertoire, self tolerance, cytokine profiles,

and different cellular and regulatory mechanisms involved) [8]. Short peptides have potential advantages over other vaccines in having physicochemical properties that enable them to present large enough amounts of pure, potent, and highly specific antigen for an immune response [9].

An effective T cell response is a critical component of any strong immune response, so putative vaccines based on peptide antigens are usually designed with a T cell recognition site within the antigen. T cells recognize the antigen in the context of a complex between the processed antigen and host MHC molecules present on the surface of antigen presenting cells (APCs). Since this recognition is based on epitope presentation by the antigen to the T cell, great care must be taken in designing the synthetic peptide as even a single amino acid change can prevent T cell recognition and drastically alter the response observed [7, 10]. Effective peptide vaccines therefore maintain their T cell receptor (TCR) repertoire [10, 11]. Due to the polymorphic nature of MHC proteins, designed T cell epitopes must also be able to be recognised by different haplotypes if an effective response is to be elicited in the majority of an outbred population [12].

An effective antibody response is dependent on the activation and proliferation of two different cell populations. B cells are activated *via* the direct interaction of cell surface anchored antibody molecules with exogenously presented antigen. Helper T-cells (T_H cells), necessary for providing appropriate cytokine help in the activation and proliferation of B cells into antibody secreting plasma cells, are themselves activated through recognition of defined antigenic epitopes in the context of MHC molecules. Interestingly, it has recently been found that a peptide vaccine lacking a T_H cell epitope can also produce functional CD8+ memory cells able to generate a protective immune response [13]. Epitope specific information is presented to antibodies on the surface of B cells [14], and constrained epitope shapes often provide better immune responses than unconstrained linear epitopes [15]. For those peptide based vaccines that target B cells for the production of antibody, it is still critical that defined helper T-cell epitopes are present for an effective cellular and humoral immune response [9].

Peptide vaccine candidates are sometimes not able to generate a sufficient CTL response, even though they induce decent antibody titres. This problem can be solved by using a poly-lysine core which eliminates the need for carrier molecules to induce a good immune response [16-19]. There is evidence that stable α-helical peptides can generate a strong immunological response, as they form more structurally defined immuno-dominant epitopes [14, 20, 21].

DESIGN OF PEPTIDE VACCINES

A classic approach to the discovery of antibody reactive peptide epitopes within a protein is called PEPSCAN analysis, first reported by Mario Geysen [22]. In the PEPSCAN approach, antbody responses to the targeted protein are analysed either from experimentally immunized animals, usually mice or from human serum samples. The sera is screened for anti-peptide antibodies against a library of synthetic peptides designed to cover multiple overlapping epitope sequences. [7, 23]. This approach can be relatively time-consuming and is usually combined with computer-based approaches.

Computer aided design of peptide vaccines has provided a useful means of capitalising on new structural insights to antigen recognition afforded by X-ray crystallography. Computational approaches allow researchers to design, analyse, and predict likely vaccine candidates *in silico*, without any benchwork. One can even predict various post-translational modifications in the viral genome [24]. Perhaps the most useful of these methods has been TEPITOPE, a matrix based algorithm developed at Hoffmann-LaRoche. TEPITOPE allows the identification of large numbers of epitopes which are recognised in large populations and show cross-reactivity between different strains of the same disease [25]. This software can be used together with cDNA microarray technology to better understand genetic specificity of a disease. It also quickly gives information of associated binding sites with the help of a virtual matrix system [26-28]. Other computational approaches include OptiMer, EpiMer [29], Gibbs sampling, and the Clustal W weight-matrix approach. All of these approaches enable predictions of likely immunogenic epitopes and results of these approaches are complementary to one another [30].

Gene knockout and signature tagged mutagenesis (STM) are also effective tools for identifying epitopes of interest. In these cases, mutants are generated either by allele replacement or by insertion and resulting functions are investigated. Large sequential and structural databases can be used to generate different antigens *in silico*. These antigens are then analyzed for immunogenic properties [24]. Another interesting approach to identifying epitopes involves phage display libraries, where selected epitopes are panned for and amplified phage clones are screened for desired properties [31, 32]. Another technique called antigenome technology identifies epitopes from the bacterial genome that present on the surface of E. *coli*. This enables prediction of antigens and validates even those not calculated by other algorithm-based approaches [33].

After key epitopes are identified by biological or computer based techniques, they are subjected to animal based trials to determine immunogenic potential with or without adjuvants. Sometimes peptides are not able to generate sufficient CTL responses or neutralizing antibody responses even after generating good antibody titers. Thus the accurate design of appropriate epitopes and selection of a suitable adjuvant are critical components of peptide vaccine discovery (see ahead). Once they have passed initial therapeutic and toxicity trials in animals, candidate peptide vaccines are investigated in clinical trials for human use.

ADVANTAGES OF PEPTIDES AS VACCINES

Synthetic peptides were first investigated as immunogens in 1963, when hapten poly-*L*-Lysine conjugates were used to generate an immune response in the study of genetic transmission in guinea pigs [34]. Synthetic peptides have the important potential advantages over other types of vaccines in being homogeneous and chemically pure (enabling safer human use), cheap and non-hazardous to synthesize, better defined immunogens that are potentially cleaner and more

specific in their interactions, and are easily stored and delivered unlike DNA-based vaccines. By contrast, vaccines based on whole organisms or cells are extremely heterogeneous mixtures of chemicals and biological materials. In the case of pneumonic plague, both the live attenuated vaccine and the dead whole cell vaccine are highly reactive mixtures and afford only weak protection, necessitating many immunizations that prevent effective use in humans [35].

Peptides can present small surfaces (epitopes) that are able to induce both effective T cell and B cell responses and can adapt to genetic changes, unlike other vaccines that face genetic restrictions due to mutations in different strains and result in complex immune responses [7]. Whereas peptides are relatively easy and non-hazardous to synthesize, many pathogens that are used as live attenuated vaccines are hazardous and difficult to culture in large quantities. Viral vector based vaccines may also cause inflammation and can induce resistance against other viruses of the same class. Some live viruses can bypass the immune responses to live virus vaccines [36]. The preferred oral route of administration of vaccines, especially to infants, is often not possible for DNA-based vaccines [37]. Other specific advantages and disadvantages of different vaccines based on viruses, tumor cells, DNA and oncoproteins have been reviewed elsewhere [4, 37-41].

PROBLEMS WITH PEPTIDES AS VACCINES

Despite some key theoretical advantages for short synthetic peptides as vaccines, only a tiny fraction of such peptides that have been evaluated in preclinical trials have progressed to Phase I clinical trials, a few to Phase II, and no short peptide vaccines have been successful in Phase III. This is undoubtedly due to a combination of problems with peptide vaccines such as low immunogenicity, chemical and conformational instability, low oral and systemic bioavailability [42], limited availability of appropriate carriers and adjuvants for effective delivery and for amplifying immune responses [9], as well as restrictions to patients of a particular HLA type [40].

Immunogenicity of peptide vaccines depends upon several factors. It is often reported that short peptides are not immunogenic, though the reasons for this may not be universally appreciated. Short peptides do not tend to exhibit much structure in water and so energy needs to be expended for them to organize into antigenic conformations that are recognized by T cells or B cells and MHC. It is generally accepted for example that peptides of six or less residues are not immunogenic, and this is likely a result of their lack of structure in solution.

Immunogenicity is almost certainly related to peptide conformation. Peptides $\leq$15 residues are rarely alpha helical and peptides $\leq$6 residues rarely adopt beta sheets or well defined turn conformations. It is now known that proteolytic enzymes responsible for peptide degradation do not process discrete alpha helices, beta sheets, or turn/loop regions of protein structure without such structures unwinding and reverting to random coils that can adopt the key beta strand that is commonly recognized by proteases [43-45]. Short peptides usually have no stable structure in water and are most often degraded in seconds to minutes by proteolytic enzymes and/or rapidly eliminated during first pass metabolism. The longer the peptide, the more stable it is to degradation and the greater its longevity, as they begin to fold into defined conformations and their buried components are afforded protection from proteolytic enzymes. In this regard, conformationally constrained epitopes appear to provide better immune responses compared to more flexible linear epitopes [15, 20, 21, 46, 47].

The chemical instability of short peptides leads to short lifetimes in the gut, bloodstream and inside cells. Both short and long peptides also exhibit poor membrane permeability, resulting in poor absorption from the gut. They have particularly low oral bioavailability, exhibit other types of metabolic instability, and often suffer from inefficient transport mechanisms [9]. An ideal vaccine must be chemically and conformationally stable enough to exist long enough for recognition by, and to induce a sustained response from, the immune system.

The development of adjuvants and carrier molecules to increase immunogenic responses to short peptides has been successful but comparatively little is still known about adjuvant biology and mechanisms of action [48]. MHC polymorphism is another factor that needs more attention during vaccine design. The presence of different MHC haplotypes between individuals complicates approaches to successful vaccination of an outbred population. However these epitopes can be used for diagnostic purposes [48].

POTENTIAL SOLUTIONS TO PROBLEMS OF PEPTIDE VACCINES

Use of an Adjuvant

To increase the immunological response to a synthetic peptide antigen, one can use an adjuvant that can either be coadministered or directly tethered to the peptide. An adjuvant can increase the immunogenic response of a peptide by improving T cell help for a peptide. Immunomodulating adjuvants can enhance peptide delivery, processing, and boost immunogenic responses. Ideally an adjuvant must be safe, non toxic, and should not induce local autoimmune responses [9, 49]. At present all adjuvants have some associated toxicities and are therefore used at low levels to avoid side effects and specific autoimmune responses. They can induce mucosal immunity, prevent antigenic competition in combination vaccines, modulate antibody avidity, and stimulate cell mediated immunity [50]. Cruz and coworkers investigated different adjuvants with the same peptide and confirmed how their effects vary under similar conditions [51].

Alum is the only co-administered adjuvant registered for human use, but many other adjuvants are in clinical trials. The understanding of adjuvant biology and their mechanisms of action are still quite limited, making it difficult to decide which adjuvant will be the most suitable for any given antigen [48]. Aluminium compounds were the first to be registered and authorized by the FDA for human use in the USA. Their preparation is fairly simple but needs rigorous control of conditions because a minor alteration in particle size can alter other physico-chemical and immunological properties. There are different structural models available for alum that act by mediating release of the vaccine from the injection

site. There are no major side effects reported to date apart from some local reactions in a few cases. Their use is limited in typhoid and influenza due to inability to induce good immune responses [52], and uncertainties about possible hazards of aluminium biology on human physiology makes their clinical use risky.

Freund's adjuvant and Montanide are other widely used adjuvants. Freund's adjuvant was discovered in 1950 [53]. Complete Freund's adjuvant (CFA) is a water-in-oil (mineral oil) emulsion that contains heat-killed mycobacteria and mannide mono-oleate, and is one of the most commonly used adjuvants today. Like other adjuvants, it produces side effects and local autoimmune responses including inflammatory disease and for these reasons is not used in human vaccines. Another preparation called incomplete Freund's adjuvant (IFA) has a composition similar to CFA except for the mycobacteria. Side effects of this preparation are less severe than for CFA. IFA is generally used along with initial booster doses. The adjuvant effects of Montanide ISA are similar to those of incomplete Freund's adjuvant (instead it has mannide oleate) with reduced side effects [54]. Other montanide preparations in trial are AS01B, AS02A, AS05, AS08 [55].

Apart from emulsions, lipids are also used in vaccine delivery in different forms. Liposomes are bilayered vesicular structures composed of phospholipids and cholesterol. These vesicles contain entrapped antigens for delivery. However, the quantity of liposomal uptake in the body can be enhanced by modifying the surface of liposome with lectin improving the efficacy level even when the vaccine is orally delivered [1]. Alternatively, a vaccine antigen could be coated with the help of a biodegradable polymer or a protective agent (microcapsules or micro-spheres). This technique is known as micro-encapsulation and requires less antigen *via* parenteral or oral routes to generate equivalent immune protection [1, 56]. Antigen release from this system depends on vaccine composition, vaccine to polymer ratio, type of polymer (poly lactic acid or poly lactic/glycolic acid i.e. PLA or PLGA), and rate of polymer degradation [57, 58]. This system produces minimal or no side effects depending on the disease type and nature of components involved in manufacture [54, 59]. Another formulation called cochleate involves trapping an antigen in a stable spiral-like structured phospholipid precipitate [1]. Other preparations incorporate lipids like lipopolysachharide [32, 54] and lipoproteins [23].

ISCOM (Immune Stimulating Complex) is a stable micelle composed of saponin, Quil A, and cholesterol held in a cage like structure approximately 35nm in size. They induce higher antibody titers when used with glycoproteins [60, 61]. A great advantage of ISCOMs is that they are orally stable, but a major drawback is the complex preparation. Another ISCOM preparation called ISCOMATRIX^{TM} contains ISCOMPREP^{TM} highly purified saponin fraction extracted from the bark of the *Quillaja saponaria* tree. This is able to induce cellular as well as humoral immune responses and is currently being evaluated for efficacy and toxicities [62-64].

Apart from adjuvants, certain carrier molecules are also used in conjugation with synthetic peptides to promote responses through more effective delivery or targetting. Tetanus toxin [65], diphtheria toxoid (DT), nontoxic mutant of diphtheria toxin, CRM197, cholera toxin CT-E29H [66], glycopolysaccharide, KLH, BSA, and OVA are most commonly used in therapeutic research. Out of these TT, DT, non-toxic mutant of diphtheria toxin are licensed for human applications. KLH (keyhole limpet cyanine) OVA, and BSA are conjugated to a peptide (generally with cysteine) before administration with/without adjuvant. These agents not only increase immunogenic levels of antigen but also help in directing these antigens to the white pulp (T and B cell zone) of splenic compartments instead of the pink pulp zone [67].

Vaccines delivered by the systemic route can cause local autoimmune or allergic reactions at the site of injection. With other vaccines it is not possible to alter such unwanted effects, but for peptide vaccines allergic reactions can often be avoided or limited by altering the isoelectric point (pI) of a peptide to neutral, thus reducing the solubility and thereby the rate of absorption without changing the immunological properties of the vaccine candidate [68].

Stabilising Peptides to Degradation

A major problem with short synthetic peptides is their chemical instability. Almost all short peptides used to date in the clinic have been linear unstructured amino acid sequences that are readily degraded by proteolytic enzymes of the gut, blood or cells, and/or rapidly metabolised by cytochrome P_{450} and other enzymes (which are now known to be present in the intestinal lining as well as the liver) resulting in short lifetimes. In addition to their instability, peptides are zwitterionic molecules that do not effectively penetrate membranes in the gut and cells, leading to poor absorption and transport problems [9]. Various methods are being developed to overcome such problems that have to date limited the utility of short peptides in medicine. One particularly promising approach to making peptides resistant to degradation by proteolytic enzymes is to constrain them either in helix [69], turn or cyclic conformations [70, 71], or beta strand mimetics with non-cleavable peptide bonds [72].

Conformational Stability

Short peptides tend to adopt random structures in water and there is an entropy penalty in organizing random peptide conformations into the single correct shape that is recognised by an MHC molecule or antibody. Stabilization of peptides in topologies (shapes) that can be recognized by immunological components would be expected to increase their affinities and promote immunogenicity. Indeed the use of different epitope specific peptides that mimic B-cell and T-cell epitope conformations have been shown to induce higher antibody responses [73]. These constrained helical epitopes are recognized by patient serum samples and are also able to generate neutralizing antibodies [47]. Calvo *et al.* also observed similar results when they reacted their helical epitope with sera from a cervical carcinoma patient [20]. Although conformationally stable peptides have shown promising immunogenicity in preclinical settings, they have not yet been fully exploited in clinical trials.

'Topological structures' called epitopes vary in the nature of immunological responses they elicit. Recognizable to-

pologies include alpha, beta, alpha-alpha, alpha-beta, alpha-beta-alpha and many other structures [74, 75]. A few studies have shown that longer peptides (>20 residues) that may adopt these structures with multiple secondary structural components can be conformationally stabilized to produce better immune responses than their linear counterparts [76-78]. Data is available to confirm that information is more effectively provided to the T cells if the peptide conformation is stable. For example, a stable α-helical structural peptide generates a higher immune response than linear analogues, as they form better immuno-dominant epitopes [14]. Conformationally constrained epitopes provide better immunity [15] and can be delivered by the oral or nasal route [66, 79]. In some cases mimotopes, which are epitopes with slightly different peptide sequences, can also be used when a particular sequence does not favour the required comformation. Mimotopes can be designed with the help of structural data available and mimic the correct conformation but have one or a few aminoacid substitutions relative to the original epitope. Mimotopes have been successfully tested against viral/bacterial/parasitic infections for induction of antibody responses in a predetermined manner [80-84].

Unfortunately, although the above information points to conformation being important for epitope recognition, there has not really been any effective take up of this idea, possibly because the technology to control conformation in short peptides of less than 20 residues has not been readily available. We now believe that new technology to mimic alpha helices [69], beta strands [72], and turns [71] makes this goal potentially feasible to achieve.

MHC Polymorphism

Each person has slightly different HLA types and thus recognises different antigens. A peptide antigen able to generate strong immunological responses in all or most individuals is a considerable challenge. MHC polymorphism is a major factor that can prevent a synthetic vaccine from showing responses in some individuals. Apart from recognising the individual it should also be able to generate specific B cell and/or T cell responses to the protein. Problems of low immune response and restriction of a vaccine to the patient of one HLA type can be solved by the use of multi-epitope peptides. This problem especially compromises viral or cancer vaccines that are intended for large populations across the globe.

A system containing a series of linear repeats of a synthetic peptide is called a multiple antigenic peptide (MAP). MAPs use peptides assembled on a lysine core through the α and ε amino acids [19, 85, 86]. Basic MAPs incorporate linear B cell and overlapping T cell epitope(s), while chimeric MAPs include B cell and non-overlapping T cell epitope(s). Both can be used to generate either monoclonal or polyclonal antibodies. Lipid modified MAPs contain mono or oligo epitopes that can be used for both cellular as well as humoral immunity applications. Macromolecular assemblies contain a mixture of these lipidated MAPs used for similar applications to overcome genetic restrictions. Constrained MAPs consist of cyclic B cell epitopes which have increased affinity for polyclonal antibodies due to their defined structure [87]. Use of a poly-lysine core appears to partly address the problem of MHC polymorphism and eliminates the need for carrier molecules to induce an appropriate immune response [16-19]. A lysine core is most commonly used because its chemical properties allow its α- amino acid and ε-amino acid to be synthetically loaded with multiple peptide antigens. Up to 8 Lys residues can be used for the purpose of stabilisation or as a carrier molecule.

Modifications to the carrier molecule can influence the speed of conjugation and dimer formation [88]. However these individual peptides can also be used as ligands for diagnosing a particular haplotype [48]. Zeng and coworkers ligated peptides by an oxime bond, thioether bond, or by disulfide bond formation between epitopes and concluded that a thioether bond between epitopes yields the highest immune response, while disulfide bonds were found to give the poorest response [89]. Villen and co-workers chemically joined peptides and demonstrated their ability to generate a neutralizing response in guinea pigs for foot-and-mouth disease [90]. Fitzmaurice and co-workers used mono, di, and tri determinant constructs from peptide sequences of influenza virus hemagglutinin and concluded that branched immunogens, especially with two B-cell determinants, stimulate T-cells at lower doses than required for monomeric T-cell determinants in mice. They also stated that the geometry of the molecule, copy number of B-cell determinants, and the choice of helper T-cell determinant, all play important roles in the effectiveness of synthetic peptide vaccines [19, 85]. Buschle and coworkers used poly-L-arginine to induce T-cell responses. They also pointed out that use of this sequence is of greater importance with peptides containing tryptophans (or other hydrophobic residues) than hydrophilic peptides. They commented on the safety of this sequence as it does not yield any measurable T cell or B cell responses by itself [91].

Multiple immunizations can cause considerable pain to infants and can overload the immune system leading to over-immunization. This may also result in one vaccine reducing the effect of another. A solution to these problems is a multi-component vaccine. But to date only two multi-component vaccines, MMR and DTP are available. Selection of one or more of the above strategies for a peptide vaccine, that can be administered containing cross-reactive antigens against 5-10 different diseases or MHC haplotypes, will be of great help in disease prevention [92].

In summary, many features need to be considered in the design of a peptide vaccine, including the structure of the B cell epitope, the sequence length of a T cell epitope, presence of amino acids (e.g. arginine, lysine) that can enhance antigen presentation, inclusion of universal T_H cell epitopes, selection of an appropriate adjuvant, the method of vaccine preparation, formulation, dosing regimen, and delivery, and the patient population to be vaccinated [38, 93].

SUMMARY OF CLINICAL TRIALS

Clinically, peptides have been trialed against many different types of cancers as well as viral, bacterial and parasitic infections. The complexity of these diseases lies in the genomic integrity of the proteins involved. Over the years, several tumor-associated antigens (TAA), defined T and B cell epitopes have gained importance in peptide vaccine studies. The importance and activities of these TAAs with/without

autoimmune responses have been reviewed recently [40, 94-101]. In the early days, peptides were investigated along with different anticancer inhibitors and in the 1990s the focus shifted towards using them with different adjuvants and cellular therapies including dendritic cells (DCs). In the late 1990s peptide vaccine trials began involving new techniques that had been used to more effectively and rationally identify better epitopes. Although some of the more promising solutions to development of short peptide vaccines are yet to be exploited, it is worth summarising some of the work conducted over the last 15 years towards the success of short peptides as vaccines.

Since 1993 there have been hundreds of short synthetic peptides examined in vaccine research, but most did not progress past preclinical (>1000) or phase I clinical (~125) trials. Few peptides progressed into phase II clinical trials (~30), and no short synthtic peptide vaccines have succeeded in passing Phase III clinical trials and become marketed for human use. Most peptides currently in Phase II clinical trials are targetted at cancer research, while treatment for viral infections is not far behind. This next section (Table **2**-**4**) attempts to exmplify mainly short peptides as vaccine candidates that have been examined in clinical trials.

Novellino *et al.* have summarized tumor-associated antigens that have been trialed as immunogens against cancer [199]. Those peptides were administered with different adjuvants or loaded on dendritic cells for immunization. Peptides from tyrosinase, gp100, HER-2/*neu* NY-ESO and HPV have been extensively studied as vaccine candidates. Table **2** presents a summary of different peptides and adjuvants that have been trialed in the clinic. Brichard *et al.* reported activity of tyrosinase peptides against CTLs on HLA-A2 melanoma [200]. Slingluff and colleagues investigated various gp100 peptides in different melanoma cell lines [201]. Later in Phase I clinical trials these peptides were studied in stage IV melanoma patients, administered with GMCSF and montanide ISA51 [148]. Recently in a Phase II randomized trial, the CTL response to these peptides with different adjuvants was measured. The immunogenic potential of the peptides (2 gp100 + 2 tyrosinase + tetanus helper peptide) administered with montanide ISA51 and GMCSF (grp1) was first compared with peptides pulsed with dendritic cells in melanoma patients (grp2)[149]. A systemic low dose of IL-2 was also administered daily to all the patients. Specific T-cell responses were measured in both SIN (Sentinel immunized node) and peripheral blood by ELIspot assay. SIN was selected for its higher sensitivity levels as compared to peripheral blood. Eighty percent of the grp1 patients had detectable CTL responses with higher response, while in grp2 only 13% of patients had a detectable CTL response. The magnitude of the CTL response was much higher with tyrosinase peptides in all cases when compared with gp100 peptides.

Cathcart and co-workers tested the efficiency of a bcr-abl derived fusion peptide vaccine against leukaemia [129]. The aim of this study was to achieve a fixed dose of peptide vaccine that could safely generate CD4+ and CD8+ responses. A further aim of this study was to investigate the efficiency of the vaccine in patients with bone marrow transplant (BMT) or on imatinib mesylate. Six fusion peptides (100μg/peptide) mixed with Quillaja Saponaria (QS-21) were administered subcutaneously on days 0, 7, 21, 35, and 54 to 14 patients with chronic myelogenous leukaemia (CML). There were no serious toxicities observed in any patients, with all 14 showing delayed hypersensitivity, 11/14 exhibiting release of IFN-γ from CD4+ cells measured by ELIspot assay, and 4/14 patients showed peptide specific IFN-γ from CD8+ cells. The most potent peptide (A3/11) induced a response with nearly a two-fold increase in IFNγ release spot cells (20SFCs/10^5 to 40SFCs/10^5 cells). The vaccine was safely administered to elicit CD4+ responses in patients with CML and BMT. However, the relationship between clinical responses and vaccination was not clearly established, for various clinical reasons. Some patients were continued on the therapy, 5 were administered interferon-alpha, with the number of patients being insufficient to establish reliable correlations.

van Driel and colleagues reported immunogenicity for HPV E7 peptides in a Phase I/II tiral [118]. They administered peptides, admixed with montanide ISA51, subcutaneously to HLA-A0201 positive advanced cervical carcinoma patients. None of the patients showed any clinical side effects. One year after vaccination 15/19 patients had progressive disease, 2/19 had stable disease and 2/19 (following chemotherapy after vaccination) had tumour regression. This suggested that the HPV peptides were not toxic and could be trialed in patients with early stage disease. Kyogoitoh *et al.* conducted a Phase I trial of various immunogens to test their safety and tolerability [190-192]. They injected different peptides from different antigenic sources mixed with montanide ISA 51 into patients with either gastric, prostate and pancreatic cancer. The peptides generally did not show any significant side effects in these patients. Only 2 of the peptides trialed showed immediate hypersensitivity reactions in pancreatic cancer patients. Gastic cancer patients showed encouraging cellular and humoral responses with grade-1 side effects.

Sha and co-workers examined the use of IL-12 as an adjuvant in HIV treatment with an HLA based C4-V3 (15res) polyvalent synthetic vaccine candidate in nine patients in a Phase I trial. The vaccine induced CTL as well as neutralizing antibody responses. All patients were administered vaccine emulsified in IFA (Montanide ISA51) administered intramuscularly (in deltoid muscle and quadriceps) at 0, 4, 8, and 16 weeks. The vaccine was generally well tolerated by 4/8 subjects. At the site of injection there were no serious side effects observed in any patient, but there were transient and mild side effects in 7/9 subjects and systemic symptoms in 3/9 subjects. Overall 6/9 subjects showed five fold increases in lymphocyte proliferation, of which 4 had three fold or greater activity above baseline on two consecutive visits to the clinic. This experiment will need to be repeated with a larger number of subjects to prove that IL-12 can indeed be used as an adjuvant in HIV therapy [203].

In another Phase I/II trial a gp41 peptide from the transmembrane protein was evaluated by Schwander *et al.* [211] for tolerance, toxicity and immunogenicity with 2.4 dinitrophenyl ficoll (DNP). The peptide was administered intradeltoid with DNP, with 11/29 patients showing increased humoral responses without side effects with the responses being increased following two booster doses.

Table 2. Clinical Trial Summary of Peptide Vaccines for Cancer

Cancer	Peptide sequence	Gene/protein Source	Adjuvant and administration	Clinical status	Ref.
Adenocarcinoma	105res Mucin peptide	5 imuunodominant epitope repeats (21aa) MUC-1 peptide	BCG, i.d.	Phase I	[102]
Breast cancer, lung cancer, and leukemia	^{235}CYTWNQMNL^{243}	WT1 gene peptide	Montanide ISA51. i.d.	Phase I	[103]
Breast cancer	106mer peptide	MUC1 peptide	KLH and IL2, s.c.	Phase I	[104]
Breast, ovarian, and prostate cancer	^{369}KIFGSSLAFL^{377}	E75- HER2/*neu* peptide	GM-CSF, i.d.	Phase I	[105-108]
Breast cancer and ovarian cancer (stage III or IV)	p369 (369-384aa)+ p688 (688-703aa)+ p971 (971-984aa	Peptides from HER2/neu protein	GM-CSF, i.d.	Phase I	[109]
Breast and ovarian cancer	ECD vaccine (p42-56 + p98-114 +p328-345) ICD vaccine (p776-790 + p927-941 + p1166-p1180), + helper peptides (p369-384 + p688-p703 + p971-984)	HER-2/neu derived peptides	GM-CSF, i.d.	Phase I	[110]
Breast cancer and ovarian cancer	^{369}KIFGSLAFLPESFDGDPA^{384} ^{688}RRLLQETELVEPLTPS^{703} ^{971}ELVSEFSRMARDPQ^{984} ^{369}KIFGSLAFL^{377} ^{689}RLLQETELV^{697} ^{971}ELVSEFRSM^{979}	HER-2/neu peptides	GM-CSF, i.d.	Phase I	[111]
Breast cancer	GVTSAPDTRPAPGSTA	MUC1 peptide	KLH+DETOX, s.c.	Phase I	[112]
Breast cancer	C-VTSAPDTRPAPGSTAPPAHG VTSAPDTRPA	MUC-1 peptide	KLH + QS-21, s.c.	Phase I	[113]
Breast cancer and lung cancer	p98-114, p369-386, p776-790, p927-941	HER2/*neu* peptides	GM-CSF, i.d.	Phase I	[114]
Breast cancer and ovarian cancer	^{369}KIFGSLAFL^{377}	Her2/*neu*	GM-CSF, i.d.	Phase I	[115]
Breast, ovarian, and colorectal tumor (advanced)	^{369}KIFGSLAFL^{377} ^{209}ITDQVPFSV^{217}	HER-2/neu gp100	IFA, s.c.	Phase I	[116]
Cervical and Vulvular intraepithelial cancer	^{11}YMLDLQPETT^{20}	HPV16 E7	IFA, i.d.	Phase I	[117]
Cervical carcinoma	^{11}YMLDLQPETT^{20} ^{86}TLGIVCPI^{93}	HPV16 E7 E7	Montanide ISA 51, s.c.	Phase I-II	[118]
Colorectal, lung, gastric, Prostate, melanoma and 13 other different cancers	14 HLA-A24 binding peptides and 16 HLA-A02 binding peptides were tested	Peptides	Monntanide ISA 51, s,c,	Phase I	[119]
Colorectal cancer(Advanced)	^{109}VYDYNCHVDL^{118} ^{315}AYIDFEMKI^{323}	SART3 SART3	s.c.	Phase I	[120]
Colorectal cancer, breast cancer, lung cancer	^{80}AYACNTSTL^{88}	Survivin2B peptide restricting HLA-A24	Peptide + PBS/HSP90/IFA, s.c.	Phase I	[121-124]

(Table 2. Contd….)

Cancer	Peptide sequence	Gene/protein Source	Adjuvant and administration	Clinical status	Ref.
Gastric carcinoma	EGPWLEEEE-peptide spacer-DT	Gastrin	Diphtheria toxoid (DT), i.m.	Phase II	[125]
Gastric cancer and cervical cancer	^{109}VYDYNCJVDL ^{315}AYIDFEMKI ^{208}HYTNASDGL ^{488}DYLRSVLEDF ^{93}DYSARWNEI ^{172}VLEGMEVV ^{246}KLVERLGAA ^{432}DLLSHAFFA ^{43}RLQEWCSVI	SART3 SART3 lCK lCK SART2 CypB lCK ppMAPkkk UBE2V	Montanide ISA51, s.c.	Phase I	[126]
Advanced malignant glioma	48 different peptides	Peptides from 12 different TAAs	Montanide ISA 51, s.c.	Phase I	[127]
Hepatocellular cancer	^{137}PLFQVPEPV^{145} ^{158}FMNKFIYEI^{166} ^{325}GLSPNLNRFL^{334} ^{542}GVALQTMKQ^{550}	hAFP peptides	Montanide-ISA51, i.d.	Phase I	[128]
Chronic myelogenous leukaemia	SSKALQRPV KQSSKALQR ATGFKQSSK HSATGFKQSSK GFKQSSKAL IVH-SATGFKQSSKALQRPVASDFEP	HLA-A0201+ HLA-A3+ HLA-A11+ HLAA-A3/11+ HLA-B8+ Class II peptide	QS-21, s.c.	Phase II	[129, 130]
Myeloid leukaemia	ATGFKQSSK + KQSSKALQR + HSATGFKQSSK + GFKQSSKAL + IVH-SATGFKQSSKALQRPVASDFEP	A11 binding A3 peptide A3 and A11 binding B8 binding b3a2-CML (2-25) peptide	QS-21 + GM-CSF. s.c.	Phase II	[131]
Lung cancer, colorectal carcinoma, and prostate cancer (except ART4$^{13-20}$)	^{690}EYRGFTQDF^{699} ^{93}DYSARWNEI^{101} ^{161}AYDLFYNYL^{169} ^{899}SYTRLFLIL^{907} ^{109}VYDYNCHVDL^{118} ^{315}AYIDFEMKI^{323} ^{84}KFHRVIKDF^{92} ^{91}DFMIQGGDF^{99} ^{208}HYTNASDGL^{216} ^{486}TFDYLRSVL^{494} ^{488}DYLRSVLEDF4^{97} ^{170}EYCLKFTKL1^{78} ^{13}AFLRHAAL^{20} ^{75}DYPSLSATDI^{84}	SART1 SART2 SART2 SART2 SART3 SART3 CyB CyB Lck Lck Lck ART1 ART4 ART4	± Estramustine phosphate, i.d.	Phase I	[132-136]
Lung cancer	^{84}KFHRVIKDF^{92} ^{91}DFMIQGGDF^{99}	CyB CyB	i.d.	Phase I	[137]
Lung cancer	^{235}CMTWNQMNL^{243}	WT1 peptide	Montanide ISA 51, i.d.	Phase II	[138, 139]
Lung cancer	^{611}EARPALLTSRLRFIPK^{626} ^{540}ILAKFLHWL^{548}	HTERT	GM-CSF, i.d.	Phase I/II	[140]

(Table 2. Contd….)

Cancer	Peptide sequence	Gene/protein Source	Adjuvant and administration	Clinical status	Ref.
Lymphoma	56 different peptides of 9-19aa	From different CDRs of tumor	IgV_H, s.c.	Phase I	[141]
Melanoma	^{209}IMDQVPFSV^{217} ^{209}ITDQVPFSV^{217} ^{368}YMDGTMSQV^{376} ^{280}YLEPGPVTV^{288} ^{86}TLGIVZPI^{93} ^{476}ILKEPVHGV^{484}	gp100 gp100 tyrosinase gp100 HPV E7 HIV RT	Montanide ISA 51, s.c.	Phase I	[142]
Melanoma	^{209}ITDQVPFSV^{217} ^{209}IMDQVPFSV^{217} ^{280}YLEPGPVTA^{288}	gp100 peptides	Estramustine phosphate, s.c.	Phase I	[143]
Melanoma	^{209}IMDQVPFSV^{217} ^{280}YLEPGPVTA^{288}	gp100 peptides	CTLA-4 + IFA, s.c.	Phase I	[144]
Melanoma	^{209}ILDQVPSFV^{217} ^{154}TKTWGQYWQV^{162} ^{457}LLDGTAATLRL^{466}	gp100 peptides	s.c.	Phase I	[145]
Melanoma	^{27}AAGIGILTV^{35}	MART	s.c.	Phase I	[146]
Melanoma	^{240}DAEKSDICTDEY^{251S} ^{369}YMDGTMSQV^{377D} ^{280}YLEPGPVTA^{288} ^{17}ALLAVGATK^{25} AQYIKANSKFIGITEL	Tyrosinase + tyrosinase + gp100 + gp100 tetanus helper peptide	GM-CSF + Montanide ISA-51 /IL-2, s.c. + i.d.	Phase II	[147-151]
Melanoma	^{26}ELAGIGILTV^{35}	Melan-A/Mart-1 peptide	Montanide AS02, s.c.	Phase I	[152]
Melanoma	MAGE-$A1_{96-104}$ MAGE-$10A_{254-262}$ $gp100_{614-622}$	Peptide	montanide ISA 51 and GM-CSF	Phase I	[153]
Melanoma (Stage III and IV)	^{26}ELAGIGILTV^{35} ^{209}IMDQVPFSV^{217} ^{368}YMNGTMSQV^{376}	MART-1+ gp100+ tyrosinase	incomplete FA +SD-9427 (progenipoietin), s.c.	Phase I	[154]
Melanoma (stage I-III)	^{209}IMDQVPFSV^{217}	gp100 (tetramer)	Montanide, s.c.	Phase I	[155]
Melanoma (Stage III and IV)	23aa peptide	MART(51-73)	Montanide AS02, s.c.	Phase I	[156]
Metastatic melanoma	^{170}VRIGHLYIL^{178}	MAGE-A12	IFA, s.c.	Phase I	[157]
Melanoma	EVDPIGHLY	MAGE-3.A1	s.c. and i.d.	Phase I	[158]
Melanoma and hepatocellular carcinoma	^{157}SLLMWITQCFL^{167} ^{157}SLLMWITQC^{165} ^{163}QLSLLMWIT^{155}	NY-ESO-1 peptide tetramers	±GM-CSF, s.c.	Phase I	[159, 160]
Melanoma	^{368}YMDGTMSQV^{376}	Tyrosinase	QS-21, i.d.	Phase I	[161]
Metastatic melanoma (stage IV)	^{243}KCDICTDEY^{251} ^{368}YMDGTMSQV^{376} ^{206}AFLPWHRLF^{214} ^{192}SEIWRDIDF^{200}	Tyrosinase Tyrosinase Tyrosinase Tyrosinase	GM-CSF, i.d.	Phase II	[162, 163]
Metastatic melanoma (stage III or IV)	^{243}KCDICTDEY^{251} ^{368}YMDGTMSQV^{376} ^{206}AFLPWHRLF^{214} ^{192}SEIWRDIDF^{200}	Tyrosinase peptides	GM-CSF ± KLH, i.d and s.c.	Phase I	[164]

(Table 2. Contd….)

Cancer	Peptide sequence	Gene/protein Source	Adjuvant and administration	Clinical status	Ref.
Metastatic melanoma	^{167}EVDPIGHLY^{175} (acetate)	MAGE-3.A1	in PBS, 2s.c. and 2i.d. sites	Phase I	[165]
Metastatic melanoma	^{26}ELAGIGILTV^{35} ^{209}IMDQVPFSV^{217} ^{368}YMNGTMSQV^{376}	MART-1 gp100 tyrosinase	IFA + SD-9427	Phase I	[154]
Metastatic melanoma	^{209}IMDQVPFSV^{217}	gp100 peptide	IFA + GM-CSF/IL-2, s.c.	Phase I	[166-168]
Resected stages III and IV melanoma	^{27}ELAGIGILTV^{35} ^{209}IMDQVPFSV^{217} ^{368}YMNGTMSQV^{376}	MART-1 + gp100 + tyrosinase	Montanide ISA51, i.v. + s.c.	Phase I	[169]
Metastatic melanoma	^{157}SLLMWITQCFL^{167} and/or 20res peptide (161-180)	NY-ESO peptides	IFA (+IL-2 where required), s.c.	Phase I	[170]
Melanoma	^{27}ELAGIGILTV^{35} ^{209}IMDQVPFSV^{217} ^{368}YMNGTMSQV^{376}	MART-1 gp100 tyrosinase	In PBS/pulsed to DC, i.v./i.n./i.d.	Phase I	[171]
Metastatic melanoma	^{209}IMDQVPFSV^{217}	gp100	Montanide ISA51/IL-2, s.c.	Phase I	[172]
Advanced Melanoma	^{27}AAGIGILTV^{35}	Melan-A	Peptide+PBMC+rIL12, s.c.	Phase I	[173]
Melanoma	^{26}EAAGIGILTV^{35} ^{1}MLLAVLYCL^{9} ^{368}YMDGTMSQV^{376} ^{280}YLEPGPVTA^{288} ^{457}LLDGTATLRL^{466}	Melan A/MART-1 Tyrosinase Tyrosinase gp100/Pmel17 gp100/Pmel17	GM-CSF, i.d.	Phase I	[174]
Melanoma	^{161}EVDPIGHLY^{169} Ac(cyA)VAAWTLKAAa	MAGE-3 PADRE965.10	IFA, s.c.	Phase I	[175]
Melanoma	$_{27}$AAGIGILTV$_{35}$	MART-1/Melan A	IFA, s.c.	Phase I	[176]
Melanoma	^{370}YMDGTMSQV^{378} ^{210}IMDQVPSFV^{218}	Tyrosinase gp100	IFA + GM- CSF, s.c.	Phase II	[177]
Melanoma	^{210}IMDQVPSFV^{218}	gp100	Montanide ISA 51 + IL-2, s.c.	Phase II	[178]
Melanoma	^{26}ELAGIGILTV^{35} ^{58}GILGFVFTL^{66}	Melan-A Influenza matrix	ASO2B, i.m.	Phase I	[179]
Advanced Melanoma	^{369}YMDGTMSQV^{377} ^{146}SSDYVIPIGTY^{156} AFLPWHRLF SEIWRDIDF ^{26}ELAGIGILTV^{35} ^{209}IMDQVPFSV^{217}	tyrosinase tyrosinase tyrosinase tyrosinase Melan-A/MART1 gp100	KLH and GM-CSF, i.d.	Phase I	[180]
Melanoma	FLWGPRALV YMDGTMSQV IMDQVPFSV YLEPGPVTVP ELAGIGILTV	MAGE 3A.2 Tyrosinase gp100 gp100 MART1	Montanide ISA 720, s.c.	Phase I/II	[181]
Melanoma	^{26}EAAGIGILTV^{35}	Melan-A	IFA, s.c.	Phase I	[182, 183]
Melanoma	^{26}EAAGIGILTV^{35}	Melan-A	IL-12, i.d.	Phase I	[184]

(Table 2. Contd….)

Cancer	Peptide sequence	Gene/protein Source	Adjuvant and administration	Clinical status	Ref.
Melanoma	^{157}SLLMWITQCFL^{167} ^{368}YMDGTMSQV^{376} ^{26}EAAGIGILTV^{35} ^{58}GILGFVFTL^{66}	NY-ESO tyrosinase Melan-A Influenza matrix	Flt3, i.d.	Phase I	[185]
Metastatic cancer	^{540}ILAKFLHWL^{548}	hTERT	IFA, s.c.	Phase I	[186]
Metastatic cancer (advanced)	^{5}KLVVVGA*V*GVGKS^{17} KLVVVGA*D*GVGKS KLVVVGA*C*GVGKS	ras peptides	Coupled to KLH + detox, i.m.	Phase I	[187]
Pancreatic cancer	$(GVTSAPDTRPAPGSTAPPAH)_5$	100 aa MUC-1 peptide	SB-AS2, i.m.	Phase I	[188]
Pancreatic adenocarcinoma	^{5}KLVVVGAGGVGKSALTI^{21}	p21 ras	GM-CSF, i.d.	Phase I/II	[189]
Pancreatic Cancer, gastric cancer and prostate cancer	30 different peptides	From different TAAs	IFA, s.c.	Phase I	[190-192]
Prostate cancer	^{369}KIFGSLAFL^{377} ^{971}ELVSEFSRM^{979} GILGFVFTL	HER-2/neu derived HER-2/neu derived Influenza matrix protein	Flt3 ligand, i.d.	Phase I	[193]
Prostate cancer	ILAKFLHWL	hTERT I540	KLH, s.c.	Phase I	[194]
Prostate cancer	Glycolipid peptide coupled to KLH	Thomsen-Friedenreich	QS21, s.c.	Phase I	[195]
Prostate cancer	^{690}EYRGFTQDF^{698} ^{109}VYDYNCHVDL^{118} ^{213}LYCESVHNF^{221} ^{248}HYRKWIKDTI^{257} ^{152}CYASGWGSI^{160}	SART1 SART3 PAP PSA PSA	Montanide ISA 51, s.c.	Phase I	[196]
Sarcoma	^{157}SLLMWITQC^{165}	NY-ESO-1,	GM-CSF, i.d.	Phase I	[197]
Synovial sarcoma	GYDQIMPKK	SYT-SSX	In PBS, s.c.	Phase I	[198]

Alzheimer's disease (AD) has been of particular interest in the context of vaccines due to its increasing global prevalence in the ageing population. AN1792 showed promising results in a Phase I safety trial in which 80 patients aged ≤85 were enrolled (two died of unrelated causes) [212]. A multicenter international Phase IIa efficacy trial of AN1792 [213] involved 372 patients receiving 0.5mL of 225μg of peptide + 50μg of QS-21 or placebo at 1, 3, 6, 9, and 12 months. Anti-AN1792 antibody titers were assessed by ELISA and MRI examinations were used to estimate changes in whole brain volume. All the patients were studied for 12 months. The trial was stopped once 18 patients developed meningoencephalitis, 16 of 18 patients received two doses, one patient received one dose, and one received three doses, 0/74 patients showed any signs of meningoencephalitis. There were no cases of meningoencephalitis reported six months after the first immunization, 12/18 patients recovered from disability, while six remained with neurologic sequelae. Meningoencephalitis developed in these patients was characterized by increased confusion, headache or lethargy. Patients were also reported to have encountered 6% reduction in whole brain volume [233]. There was no direct correlation established between serum IgG levels as they were not detectable in 3/18 patients, while another 15 had about 25% more than expected based on pre-clinical data. T cells were also suspected as Aβ42 contains one T-cell activating domain. Another possible explanation could be that clearance of amyloid from the brain might trigger this inflammatory process, Aβ42 vaccination being associated with microglial activation that is inactivated after 9 months of immunization. The presence of Aβ42 in neuritic plaques that characterise AD is now generally thought to be a consequence and not a cause of disease [234].

Vandenbark and coworkers have reported several clinical trials for different peptides against multiple sclerosis. These peptides were administered with IFA and their T cell responses were measured. When vaccinated with BV5S2 and BV6S1 peptides patients showed diminished IL-10 secretion and restored TCR response in a Phase I trial [235]. Other trials conducted on multiple sclerosis, rheumatoid arthritis and psoriasis vulgaris since 1991 have been summarized elsewhere [223].

Table 3. Clinical Trial Summary of Peptide Vaccines for HIV

Viral infection	Peptide	Peptide source	Adjuvant and administration	Clinical status	Ref.
HIV	15aa sequence from V3 loop of six HIV-1 isolates	Multiepitope TAB9 peptide	ISA 720, i.m.	Phase I	[202]
HIV	C4-V3 vaccine	15aa from fourth constant region and an epitope from third variable region HLA-B7 restricted	IFA +IL-12, s.c.	Phase I	[203]
HIV	aa295-325	gp120 from V3 region	± Alum, i.m.	Phase I	[204-206]
HIV	Two peptides 39res and 42res	PCLUS 3-18MN and PCLUS 1-18MN Envelop peptides	Montanide ISA 51, s.c	Phase I	[207]
HIV	Nef (66-97), Nef (117-147), Nef(182-205), Gag (183-214), Gag (253-284), Env (303-335)	6 lipopeptides	Lipopeptide + QS21, i.m.	Phase I	[208]
HIV	HGP-30	HIV p17	KLH + alum, i.m.	Phase I	[209, 210]
HIV	gp41 peptide	From gp41 transmembrane protein	2.4 dinitrophenyl-Ficoll (F46), intradeltoid	Phase I/II	[211]

FINAL COMMENTS

Vaccines certainly proved to be highly effective in the 20th Century for the prevention of diseases ranging from basic bacterial infections to the most common viral infections, and are currently in development to treat some of the most important diseases of modern times like AIDS, influenza, cancer, malaria and Alzheimer's disease. Development of new vaccines has been facilitated by the recent availability of new biotechnologies, enabling creation of DNA-based vaccines, cellular vaccines, sub-unit vaccines, live/attenuated vaccines, and peptide vaccines.

As prospective vaccines, synthetic peptides are non-hazardous and cheaper to produce, purer and free of the contaminants of traditional vaccines, and easier to handle, store and transport. No biological organisms are required for their production. Peptides can be as efficacious as other vaccines, as demonstrated in some early stage clinical trials, but peptides have not yet come to market despite their introduction to animals as potential vaccine candidates four decades ago. Most clinical trials (80%) on peptides over the last 12 years have only commenced in the last five years, indicating that it is too early to judge recent clinical progress in the area.

Table 4. Clinical Trial Summary of Peptide Vaccines for Other Diseases

Pathogen/disease	Peptide	Peptide source	Adjuvant and administration	Clinical status	Ref
Alzheimer	Aβ(1-42)	Amyloid precursor protein	QS-21, interadeltoid	Phase IIa (stopped)	[212, 213]
Bee Venom allergy	^{45}GESKHGLTNTASHTRLSC^{62} ^{82}YFVGKMYFNLI^{92} ^{113}RCLHYTVDKSKP^{124}	PLA2	s.c.	Phase I	[214]
Cat allergy	6peptides + 5peptides	Fel d 1 peptides of Chain1+chain2	i.d.	Phase I	[215, 216]
D. *pteronyssinus*	^{117}CGIYPPNANKIREALAQTHSA^{133}	Der p 1	Coupled to virus like particles, i.m. or s.c.	Phase I	[217]
Enterotoxic E *coli*	44aa	58-83 of porcine heat labile B subunit + 18aa of heat stable segment	Oral	Phase I	[218]

(Table 4. Contd….)

Pathogen/disease	Peptide	Peptide source	Adjuvant and administration	Clinical status	Ref
Multiple sclerosis	^{39}LGQGPEFLTYFQNAEQLESK^{58}	TCRVβ6.5	IFA, i.m.	Phase I	[219, 220]
Multiple sclerosis	NH_2-LGQGPEFLTYFQNEAQLKS-COOH	20aa sequence derived from CDR2 region of BC6S2/6S5 B chain of TCR	i.m.	Phase I	[221]
Multiple sclerosis	^{38}ALGQGPQFIFQTYEEEERQRG^{58} ^{38}LGQGPEFLIYFQGTGAADDSG^{58}	IL-10		Phase I	[222]
Multiple sclerosis	BV5S2 + BV3 + BV9 + BV12S2	CDR2 peptides	IFA	Phase I	[223]
Multiple sclerosis	BV5S2 (Y49T) + BV6S5 + BV13S	CDR2 peptides	IFA	Phase II	[223]
Multiple sclerosis	Vβ5.2$^{39\text{-}59}$ and Vβ6.1$^{39\text{-}59}$	V (CDR2) region of TCR	Ringer soln. i.d.	Phase I	[56]
Multiple sclerosis	^{83}ENPWHFFKNIVTPRTP^{99}	Myelin basic protein	s.c.	Phase II	[224]
Multiple sclerosis	^{38}ALGEGPEFIFETYQQQQRERG^{58} ^{39}LGEGPQFLTYFENQAELQKS^{58} ^{42}GLRLIHYSVGAGITDEGQV^{60}	BV5S2 BV6S5 BV13S1	IFA, i.d.	Phase I/II	[225]
Multiple sclerosis (IRC)	BV5S2+BV6S1	CDR2 peptides	IFA	Phase I	[223]
Multiple sclerosis (IRC)	BV5S2	CDR2 peptide	IFA	Phase I/II	[223]
P. *falciparum*	102mer peptide	Pf CS peptide (283-383)	alum and montanide-ISA720, i.m.(deltoid muscle)	Phase I	[226]
P.*falciparum*	NANPNANPNANP- T cell epitope DPNANPNVDPNANPNV- B cell epitope	4 - Identical epitopes from circumsporozoite (CS) protein	Emulsified with alum, s.c.	Phase I	[227, 228]
P.*falciparum*	48ressidue peptide	Synthetic polyoxime complex containing universal T cell epitope from CS protein and B cell epitope,	Lipopeptide P3C (endogenous adjuvant), s.c.	Phase I	[229]
Psoriasis vulgaris (IRC)	BV3, BV13S1	CDR2 peptide cocktail	IFA	Phase II	[223]
Rheumatoid arthritis	20aa peptide	HLA-DR4	Alum, i.m.	Phase I	[230]
Rheumatoid arthritis	SQIVNDFQKGDIAEGYS	Vβ17 (Vβ8.2 in mice) from CDR2	IFA, i.m.	Phase I	[231]
Rheumatoid arthritis (IRC)	BV17+ BV14+ BV3	CDR2 peptide cocktail	IL-10/IFA, i.m.	Phase II	[223, 232]
Rheumatoid arthritis (IRC)	BV17, BV14, BV3	CDR2 peptide	IFA	Phase II	[223]

Promising recent developments in more rapid and efficient methods of identifying viable epitopes of immunogenic polypeptides/proteins, increased chemical stability of short peptides, greater stabilization of structural shapes in short peptides, new methods of amplifying immunogenicity, and improved bioavailablity of peptide mimetics have yet to be fully exploited in the development of synthetic peptide vaccines and we can therefore expect that significant clinical progress might be made over the next decade in the development of more effective small peptide vaccines.

A number of questions remain unanswered about the future of peptides as vaccines. Can the problems of peptide vaccines such as chemical and conformational stability and

methods of delivery now be solved? Is co-administration of, or complexation with, adjuvants or carriers absolutely necessary and, if so, can much safer and more effective adjuvants and carriers be developed? Will presentation of peptides in defined, stable conformations and in chemically/metabolically stable forms be advantageous for vaccine effectiveness? Is presentation of multiple epitopes in synthetic peptides necessary to provide effective induction of immune protection? Epitope-based vaccines do seem to show promise for the treatment of different malignancies in early clinical trials and thus we believe that short peptides hold a great deal of promise as future candidate vaccines.

In summary, considerable effort has been invested in identifying factors that affect vaccine properties of short peptides, and challenging problems still remain in obtaining potent and sustained immunogenicity, and in enhancing delivery, chemical stability, and conformational stability of short peptide vaccines. All these issues have severely limited therapeutic uses of short peptides as vaccines in the past, but there has been substantial progress made in recent years towards solving each of these problems and those advances have yet to translate into new peptide vaccines. New developments in the future may include single dose vaccines that are able to autoboost after a time, perhaps through controlled slow release delivery systems [236], which would be ideal vaccine candidates for the 21st century. Vaccines that have efficacy against multiple diseases may also become available and would be of great interest especially in the developing world. Additionally, peptides can serve other useful roles in the clinic such as being used as biomarkers to monitor the progress of diseases [237]. In time, new generations of synthetic peptide vaccines may fulfill many of these expectations.

ABBREVIATIONS

APCs = Antigen presenting cells

ART-4 = Adenocarcinoma antigen recognized by T cells 4

bcr-abl = Breakpoint cluster region- abelson

CDK = Cyclin-dependent kinase

CEA = Carcinoembryonic antigen

CFA = Complete Freund's adjuvant

CML = Chronic myeloid keukaemia

CTLs = Cytotoxic T cells

CypB = Cyclophilin B

DC = Dendritic cell

GMCSF = Granulocyte macrophage colony stimulating factor

Gp100 = Glycoprotein 100kDa

hAFP = Human alpha fetoprotein

HER-2/*neu* = Human epidermal receptor-2/neurological

HPV = Human papilloma virus

hTERT = Human telomerase reverse transcriptase

IFA = incomplete Freund's adjuvant

MAGE = Melanoma antigen

MAP = Multiple antigenic peptide

MART-1 = Melanoma antigen recognized by T cells-1

MHC = Major histocompatible cells

MVF = Measles virus fusion protein

NY-ESO = New York esophageous

PADRE = Pan DR epitope

PSA = Prostate-specific antigen

SART = Squamous antigen rejecting tumor

SCC = Squamous cell carcinoma

SSX = Synovial carcinoma, X breakpoint 2

TAA = Tumor associated antigens

TCR = T cell receptor

WT = Wilm's tumor gene

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