

COMMENTARY

Recombinant and epitope-based vaccines on the road to the market and implications for vaccine design and production

Patricio Oyarzún^a and Bostjan Kobe^b

^aBiotechnology Center, Facultad de Ingeniería y Tecnología, Universidad San Sebastián, Concepción, Chile; ^bSchool of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience and Australian Infectious Diseases Research Center, University of Queensland, Brisbane, Australia

ABSTRACT

Novel vaccination approaches based on rational design of B- and T-cell epitopes - epitope-based vaccines - are making progress in the clinical trial pipeline. The epitope-focused recombinant protein-based malaria vaccine (termed RTS,S) is a next-generation approach that successfully reached phase-III trials, and will potentially become the first commercial vaccine against a human parasitic disease. Progress made on methods such as recombinant DNA technology, advanced cell-culture techniques, immunoinformatics and rational design of immunogens are driving the development of these novel concepts. Synthetic recombinant proteins comprising both B- and T-cell epitopes can be efficiently produced through modern biotechnology and bioprocessing methods, and can enable the induction of large repertoires of immune specificities. In particular, the inclusion of appropriate CD4+ T-cell epitopes is increasingly considered a key vaccine component to elicit robust immune responses, as suggested by results coming from HIV-1 clinical trials. *In silico* strategies for vaccine design are under active development to address genetic variation in pathogens and several broadly protective “universal” influenza and HIV-1 vaccines are currently at different stages of clinical trials. Other methods focus on improving population coverage in target populations by rationally considering specificity and prevalence of the HLA proteins, though a proof-of-concept in humans has not been demonstrated yet. Overall, we expect immunoinformatics and bioprocessing methods to become a central part of the next-generation epitope-based vaccine development and production process.

ARTICLE HISTORY

Received 3 August 2015
Revised 27 August 2015
Accepted 10 September 2015

KEYWORDS

CD4+ T-cell epitopes; epitope-based vaccines; immunoinformatics; in-silico vaccine design; recombinant protein vaccines

Introduction

Vaccines have decreased the morbidity and mortality of a large number of infectious diseases, including major global killers such as smallpox and poliomyelitis. However, for several diseases causing significant public health impact vaccines are not yet available.¹ Conventional vaccination strategies, based on inactivated or live attenuated viruses, are strain-specific and their efficacy usually depends on neutralizing antibodies. By contrast, novel vaccination approaches based on rational design of B- and T-cell epitopes (epitope-based vaccines) promise to induce large repertoires of immune specificities, as well as to deal more effectively with genetic variation both in pathogens and humans. Epitope-based vaccines can be either minimal-length epitopes, which generally suffer from poor immunogenicity, or longer peptides composed of multiple epitopes (multi-epitope peptide vaccines), either based on linear arrangements or branched/dendrimeric structures such as multiple-antigenic peptides.²

The malaria RTS,S vaccine

Currently, epitope-based vaccines have not yet reached the market, which contrasts with the steadily growing peptide drug market, with more than 60 therapeutic peptides commercially available.³ However, the research and development of epitope-based vaccines has gained significant momentum in

pharmaceutical industry during the past decade, owing to the progress made on methods such as recombinant DNA technology, advanced cell-culture techniques, immunoinformatics and rational design of immunogens. Accordingly, a milestone could be reached by the end of 2015 when the WHO makes the final recommendation on the next-generation Malaria RTS,S vaccine (MosquirixTM), which has been recently approved by the European Medicines Agency for the use in African children at risk of the disease.⁴ The RTS,S was developed over 2 decades and tested in multiple experimental and field trials,⁵ being the first candidate malaria vaccine to reach phase-III clinical trials (more than 15,000 infants and children participated in 7 sub-Saharan African countries),⁶ and will be potentially the first licensed vaccine against a parasitic disease in humans. The RTS,S vaccine is an epitope-focused recombinant protein-based vaccine based on the hepatitis B surface antigen (HBsAg) virus-like particle (VLP) platform, which was genetically-engineered to include the C-terminal region of the *Plasmodium falciparum* circumsporozoite protein (CSP), the predominant surface antigen involved in the attachment of the parasite to liver cells (pre-erythrocytic stage).⁷ The region of the CSP included in the RTS,S vaccine comprises a number contiguous immunogenic epitopes: (i) a 4-amino-acid (NANP) amino acid repeat sequence that defines an immunodominant B-cell epitope;

(ii) a highly variable CD4+ T-cell epitope; (iii) a highly variable CD8+ T-cell epitope and (iv) a conserved “universal” CD4+ T-cell epitope at the C-terminus.⁸ Its success is a significant achievement and demonstrates that a recombinant protein vaccine containing only “isolated” B- and T-cell epitopes from a single protein (CSP) delivered on a heterologous carrier can elicit significant protection in humans. Anti-NANP antibodies play a central role in inducing protection by preventing the parasite from infecting the liver and thus blocking progression to red blood cells and clinical malaria.⁹ Although the vaccine is only partially effective, it could still benefit millions of children by reducing the disease burden.¹⁰ VLPs are produced in yeast cells (*Saccharomyces cerevisiae*) by co-expressing the CSP fragment (epitopes)–HBsAg fusion protein plus the free HBsAg (S-antigen).

“Universal” influenza virus vaccines

Although not an epitope-based vaccine, FluBlok[®] represents an outstanding next-generation vaccine, the world’s first (trivalent) protein-based influenza vaccine developed with modern recombinant DNA technology, which was approved in 2013 by the US Food and Drug Administration (FDA) for the prevention of seasonal influenza disease in people 18–49 years of age. Unlike the whole-virus based vaccine, which is produced in eggs using technology that is more than 60 years old,¹¹ FluBlok[®] is produced using the baculovirus-insect cell expression system.¹² In the event of an influenza pandemic or vaccine supply shortage, this manufacturing bioprocess has the potential to start up vaccine production much faster, because the production is not dependent on an egg supply or on availability of the influenza virus. The antigens included in FluBlok[®] correspond to full-length recombinant haemagglutinin protein (rHA), including the transmembrane domain, the HA1 (stem domain) and HA2 regions (globular head domain). The primary target of neutralizing antibodies that confer protective immunity against influenza viruses are variable immunodominant regions in close proximity to the receptor-binding site at the HA globular head. To cope with antigenic variation of the virus, the vaccine contains rHA proteins accounting for 2 influenza virus A strains (H1N1 and H3N2) and one influenza virus B strain. However, the immune protection conferred by neutralizing antibodies is still highly specific to circulating influenza virus strains or subtypes.

An active area of research on epitope-based influenza vaccines aims to develop broadly protective “universal” vaccines based on conserved protein regions or peptides (B- and T-cell epitopes) that are shared by all strains. Some of these vaccines are expected to enter phase-II and III clinical trials in the coming years, shedding light on whether these next-generation concepts are able to offer increased cross-reactivity against multiple influenza strains.¹³

A major advantage of polypeptide vaccines is the inclusion of both the B- and T-cell epitopes in the same formulation. Accordingly, cellular immune responses seem to play an important role in the cross-protective immune response against influenza virus and might be a crucial addition to current antibody-inducing influenza vaccines.¹⁴ An advanced-stage influenza vaccine embodying this concept is Multimeric-001[®],

which is a “universal” vaccine containing 9 conserved linear epitopes (both B- and T-cell epitopes) from HA, nucleoprotein and matrix protein-1. The epitopes are linearly arranged in triplicates and combined into a single recombinant 50-kDa synthetic protein that is produced in *E. coli* using standard fermentation and purification methods. The vaccine company developer (BiondVax) is currently planning phase-III studies,¹⁵ after completing phase-II clinical trials where Multimeric-001[®] proved to be safe and immunogenic in humans and successfully stimulated both humoral and cellular immune responses against a wide variety of influenza A and B strains.¹⁶ Readers should note that Multimeric-001[®] has not yet been proven clinically to be a universal vaccine; the immunological data are supportive but its universality is yet to be proven.

Another promising influenza virus antigen is the matrix protein 2 (M2), a transmembrane protein that acts as a proton-selective ion channel and plays a crucial role in helping release the genetic material of the virus into the host cell. The ectodomain of the matrix protein 2 (termed M2e) is a potential target to design a universal influenza vaccine, as it consists of a 23 amino-acid peptide containing the B-cell linear epitope that is highly conserved across influenza virus subtypes.¹⁷ Phase-I/II clinical trials with the M2e-peptide vaccine candidates have been successfully completed using a recombinant fusion protein (produced in *E. coli*) that links 4 tandem copies of the M2e peptide antigen to *Salmonella* Typhimurium flagellin and a TLR5 ligand (adjuvant).¹⁸ The influenza virus HA stalk domain is also an attractive target for the induction of a cross-reactive humoral response, as it is more conserved than the head domain. However, vaccines based on the HA stalk-reactive antibodies have yet to enter the clinical phase.¹⁹

Dealing with HIV-1 genetic diversity

From a peptide-vaccine design point of view, there is much room for novel developments and improvements coming from the bioinformatics field of immunoinformatics, thanks to the development of numerous computational algorithms aiming to improve the vaccine coverage both in terms of viral strains and subtypes,²⁰ as well as in terms of the target population.²¹ For example, while the previously described influenza virus vaccines are based on conserved epitopes, the development of a universally effective HIV-1 vaccine becomes a formidable challenge, given that this virus displays the most genetic diversity of any virus studied to date.²² Thus, the generation of HIV-1 envelope glycoprotein (Env) immunogens that elicit neutralizing antibodies against circulating HIV-1 strains is a major goal of HIV-1 vaccine development.²³ Env proteins on the virion surface are trimeric spikes comprising the gp120 receptor-binding subunit and the gp41 fusion subunit. During the past 3 decades of HIV vaccine research, only 3 candidate vaccines have completed phase-III clinical trials with moderate success, reflecting the need for additional research and development of innovative approaches.²⁴ In 2009, a Phase-III trial known as RV144 was completed in Thailand, which assessed a “prime-boost” combination of 2 vaccines: ALVAC[®] HIV vaccine (the prime; a viral vector expressing genetically engineered versions of Gag, Env, and Pol proteins) and AIDSVAX[®] B/E vaccine (the boost; a bivalent gp120 envelope protein vaccine).

This study provided the first evidence that an HIV vaccine can provide protective efficacy against HIV acquisition. However, its modest achievement emphasized the importance of generating robust humoral and cellular responses against the virus,²⁵ and raised the question whether CD4+ T-cell responses may represent a beneficial component of an efficacious HIV vaccine.²⁶ Accordingly, the discovery and inclusion of appropriate CD4+ T-cell epitopes is considered paramount in epitope-based vaccine design, because cognate help provided by CD4+ T-cells is essential for the generation of vigorous humoral and cellular responses by promoting optimal expansion of CD8+ cytotoxic T-cells, generating and maintaining T-cell memory and promoting B-cell differentiation.²⁷ This necessity is underscored by the disappointing results of a large phase-II clinical trial of an HIV vaccine that elicited only CD8+ cytotoxic T-cell responses unable to prevent HIV-1 infection,²⁸ and a phase-III clinical trial of an HIV vaccine that stimulated an antibody response also did not correlate with the incidence of HIV-1 infection.²⁹ In addition, the critical role of CD4+ T-helper cells in effective HIV-specific immunity has been documented using HIV-infected individuals where loss of these cells was noted as the hallmark symptom of disease.³⁰

Another issue potentially accounting for the limited success in these major trials is the small number of epitopes contained in the vaccines, which could be insufficient to induce an effective protection against a wide range of global HIV isolates.³¹ Interestingly, a phase-I human vaccine trial of a novel polypeptide vaccine of HIV CD4+ T-cell epitopes (EP-1043) and a DNA vaccine encoding CD8+ T-cell epitopes demonstrated that the vaccine is safe, well-tolerated, and immunogenic.³² EP-1043 is a synthetic recombinant protein designed with 18 CD4+ T-cell epitopes derived from conserved sequences among HIV isolates of multiple clades, which are separated by glycine-proline-based (GPGPG) spacers to enhance proteolytic cleavage between individual epitopes. The gene segment encoding the vaccine was produced synthetically using overlapping oligonucleotides, while the protein was expressed in insect cells using a baculovirus expression system. In addition, the DNA vaccine encoded 21 HIV-derived CD8+ T-cell epitopes plus the promiscuous PADRE CD4+ T-cell epitope.^{33,34}

Considerations for epitope-based vaccine design

To address the issue of HIV genetic variation and immune escape, the genetic algorithm-based “mosaic method” was developed to generate artificial composite protein sequences (polyvalent mosaic proteins) that are optimized to include a maximal diversity of putative T-cell epitopes.³⁵ Instead of searching conserved sequences within highly variable HIV-1 viral proteins, the method optimizes natural antigen sequences to increase the cross-reactivity of vaccine responses for diverse HIV-1 isolates. This approach has been able to achieve between 74% and 87% coverage of HIV-1 Gag sequences, whereas a single natural Gag protein achieved only 37% to 67% coverage.³⁶ HIV-1 mosaic antigens have been shown to be processed and expressed by human T cells *in vitro*³⁷ and several proof-of-concept immunogenicity studies in non-human primates have demonstrated that vector-encoded HIV mosaic antigens improve the depth and breadth of cellular immune responses,

as well as antibody responses.³⁸ In particular, mosaic HIV Env vaccines delivered in adenovirus or by modified vaccinia Ankara (MVA), a replication-deficient viral vector, demonstrated a strong protective effect against infection by a subsequent simian human immunodeficiency virus challenge.³⁹ Currently, a phase-I clinical trial is recruiting participants to assess the safety and tolerability of MVA-HIV Env mosaic vaccine in healthy adult participants.

A second major issue to deal with in genetically heterogeneous human populations is the human leukocyte antigen (HLA) restriction of the targeted subjects. A number of immunoinformatics algorithms have been developed to guide the selection of T-cell epitopes that maximize the fraction of individuals potentially covered by multi-epitope peptide vaccines, including supertype-based and allele-based epitope selection methods.⁴⁰ Supertypes are pre-defined clusters of HLA molecules sharing overlapping peptide repertoires,⁴¹ which allow the selection of promiscuous epitopes potentially ensuring broad population coverage. Supertype-based selection methods include Pevvac⁴² and Multipred2.⁴³ By contrast, allele-based selection methods define promiscuous epitopes as those restricted to as many HLA alleles as possible in the target population, as a function of specific allele frequency distributions. These methods include OptiTope,⁴⁴ Episopt,⁴⁵ and Predivac-2.0.²¹ In addition, some algorithms have been proposed to simultaneously optimize coverage of HLA alleles (target population) and pathogen antigenic coverage.⁴⁶⁻⁴⁸ Other methods for epitope-based vaccine design focus on the vaccine assembly stage of linear polypeptide constructs, aiming at improving T-cell epitope processing and to minimize junctional “neoepitopes.”⁴⁹

Conclusions and perspectives

Peptide antigens produced through chemical synthesis - synthetic peptides - are advantageous from a manufacturing point of view. However, progress made on clinical testing and production of epitope-based immunogens by recombinant and advanced biotechnological methods is driving the development of new and more effective vaccines. This is (i) owing to the versatility of these systems to genetically arrange large combinations of B- and T-cell epitopes capable of eliciting robust humoral and cellular immune responses; and (ii) to support a more prominent role of cell-mediated immune responses to address the lack of immunogenicity that may be related to an insufficient stimulation of CD4+ T-helper cells. In our opinion, modern recombinant DNA and bioprocessing technologies, such as the baculovirus-insect cell expression system, are going to dominate the development of novel and more effective epitope-based vaccines, owing to the production and purification procedures that can be carefully designed to obtain high yields of a well-defined product in a cost-effective manner. Regarding vaccine design, despite immunoinformatics and *in silico* strategies remaining still at a development stage, even moderate successes might lead to significant progress on epitope-based vaccine efficacy against highly variable pathogens, especially in terms of optimizing the T-cell epitope arrangement in the polypeptide construct (vaccine assembly) to improve cellular processing and epitope presentation to T-cells, by providing

multi-strain protection against highly variable viral pathogens (“universal” vaccines) and improving coverage in the target populations by rationally considering specificity and prevalence of the HLA proteins.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The work in the authors’ laboratories is supported by a National Health and Medical Research Council (NHMRC) Program grant 1071659 to BK. BK is a National Health and Medical Research Council (NHMRC, Australia) Research Fellow (1003326).

References

- [1] Koff WC, Burton DR, Johnson PR, Walker BD, King CR, Nabel GJ, Ahmed R, Bhan MK, Plotkin SA. Accelerating next-generation vaccine development for global disease prevention. *Science* 2013; 340:1232910; PMID:23723240; <http://dx.doi.org/10.1126/science.1232910>
- [2] Black M, Trent A, Tirrell M, Olive C. Advances in the design and delivery of peptide subunit vaccines with a focus on Toll-like receptor agonists. *Expert Rev Vaccines* 2010; 9:157-73; PMID:20109027; <http://dx.doi.org/10.1586/erv.09.160>
- [3] Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. *Drug Discov Today* 2015; 20:122-8; PMID:25450771; <http://dx.doi.org/10.1016/j.drudis.2014.10.003>
- [4] Malaria vaccine candidate has demonstrated efficacy over 3-4 years of follow-up. <http://www.maliarivaccine.org/pr2015Apr24-RTSS.php>, 2015
- [5] RTS S/CTP. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; 386:31-45; PMID:25913272; [http://dx.doi.org/10.1016/S0140-6736\(15\)60721-8](http://dx.doi.org/10.1016/S0140-6736(15)60721-8)
- [6] Tinto H, D’Alessandro U, Sorgho H. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; 386:31-45; [http://dx.doi.org/10.1016/S0140-6736\(15\)60721-8](http://dx.doi.org/10.1016/S0140-6736(15)60721-8)
- [7] Whitacre DC, Espinosa DA, Peters CJ, Jones JE, Tucker AE, Peterson DL, Zavala FP, Milich DR. P. falciparum and P. vivax epitope-focused VLPs elicit sterile immunity to blood stage infections. *PLoS One* 2015; 10:e0124856; PMID:25933001; <http://dx.doi.org/10.1371/journal.pone.0124856>
- [8] Crompton PD, Pierce SK, Miller LH. Advances and challenges in malaria vaccine development. *J Clin Invest* 2010; 120:4168-78; PMID:21123952; <http://dx.doi.org/10.1172/JCI44423>
- [9] Moorthy VS, Ballou WR. Immunological mechanisms underlying protection mediated by RTS,S: a review of the available data. *Malaria J* 2009; 8:312; <http://dx.doi.org/10.1186/1475-2875-8-312>
- [10] Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmüller B, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Eng J Med* 2011; 365:1863-75; <http://dx.doi.org/10.1056/NEJMoa1102287>
- [11] Perdue ML, Arnold F, Li S, Donabedian A, Cioce V, Warf T, Huebner R. The future of cell culture-based influenza vaccine production. *Expert Rev Vaccines* 2011; 10:1183-94; PMID:21854311; <http://dx.doi.org/10.1586/erv.11.82>
- [12] Cox MM, Hollister JR. FluBlok, a next generation influenza vaccine manufactured in insect cells. *Biologicals* 2009; 37:182-9; PMID:19297194; <http://dx.doi.org/10.1016/j.biologicals.2009.02.014>
- [13] Gottlieb T, Ben-Yedidia T. Epitope-based approaches to a universal influenza vaccine. *J Autoimmunity* 2014; 54:15-20; <http://dx.doi.org/10.1016/j.jaut.2014.07.005>
- [14] Soema PC, van Riet E, Kersten G, Amorij JP. Development of cross-protective influenza vaccines based on cellular responses. *Frontiers Immunol* 2015; 6:237; <http://dx.doi.org/10.3389/fimmu.2015.00237>
- [15] Morrison N. BioPharm Insight: BiondVax mulls partnership, funding and CMO options for universal flu vaccine Phase III – CEO. 2015. Available at <http://www.biondvax.com/2015/07/02/biopharm-insight-biondvax-mulls-partnership-funding-and-cmo-options-for-universal-flu-vaccine-phase-iii-ceo/>
- [16] Atsmon J, Kate-Ilovitz E, Shaikevich D, Singer Y, Volokhov I, Haim KY, Ben-Yedidia T. Safety and immunogenicity of multi-mer-001—a novel universal influenza vaccine. *J Clin Immunol* 2012; 32:595-603; PMID:22318394; <http://dx.doi.org/10.1007/s10875-011-9632-5>
- [17] Ebrahimi SM, Tebianian M. Influenza A viruses: why focusing on M2e-based universal vaccines. *Virus Genes* 2011; 42:1-8; PMID:21082230; <http://dx.doi.org/10.1007/s11262-010-0547-7>
- [18] Turley CB, Rupp RE, Johnson C, Taylor DN, Wolfson J, Tussey L, Kavita U, Stanberry L, Shaw A. Safety and immunogenicity of a recombinant M2e-flagellin influenza vaccine (STF2.4xM2e) in healthy adults. *Vaccine* 2011; 29:5145-52; PMID:21624416; <http://dx.doi.org/10.1016/j.vaccine.2011.05.041>
- [19] Steel J, Lowen AC, Wang TT, Yondola M, Gao Q, Haye K, García-Sastre A, Palese P. Influenza virus vaccine based on the conserved hemagglutinin stalk domain. *Mbio* 2010; 1:e00018-10; PMID:20689752
- [20] Sirskyj D, Diaz-Mitoma F, Golshani A, Kumar A, Azizi A. Innovative bioinformatic approaches for developing peptide-based vaccines against hypervariable viruses. *Immunol Cell Biol* 2011; 89:81-9; PMID:20458336; <http://dx.doi.org/10.1038/icb.2010.65>
- [21] Oyarzun P, Ellis JJ, Gonzalez-Galarza FF, Jones AR, Middleton D, Boden M, Kobe B. A bioinformatics tool for epitope-based vaccine design that accounts for human ethnic diversity: application to emerging infectious diseases. *Vaccine* 2015; 33:1267-73; PMID:25629524; <http://dx.doi.org/10.1016/j.vaccine.2015.01.040>
- [22] Ndung’u T, Weiss RA. On HIV diversity. *AIDS* 2012; 26:1255-60; PMID:22706010; <http://dx.doi.org/10.1097/QAD.0b013e32835461b5>
- [23] Montefiori DC, Mascola JR. Neutralizing antibodies against HIV-1: can we elicit them with vaccines and how much do we need? *Curr Opin HIV AIDS* 2009; 4:347-51; PMID:20048696; <http://dx.doi.org/10.1097/COH.0b013e32832f4a4d>
- [24] Sanou MP, De Groot AS, Murphey-Corb M, Levy JA, Yamamoto JK. HIV-1 vaccine trials: evolving concepts and designs. *Open AIDS J* 2012; 6:274-88; PMID:23289052; <http://dx.doi.org/10.2174/1874613601206010274>
- [25] Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Eng J Med* 2009; 361:2209-20; <http://dx.doi.org/10.1056/NEJMoa0908492>
- [26] Streeck H, D’Souza MP, Littman DR, Crotty S. Harnessing CD4(+) T cell responses in HIV vaccine development. *Nat Med* 2013; 19:143-9; <http://dx.doi.org/10.1038/nm.3054>
- [27] Rosa DS, Ribeiro SP, Cunha-Neto E. CD4+ T cell epitope discovery and rational vaccine design. *Arch Immunol Ther Exp* 2010; 58:121-30; <http://dx.doi.org/10.1007/s00005-010-0067-0>
- [28] Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 2008; 372:1881-93; PMID:19012954; [http://dx.doi.org/10.1016/S0140-6736\(08\)61591-3](http://dx.doi.org/10.1016/S0140-6736(08)61591-3)
- [29] Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobs DV, Popovic V, Self SG, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. *J Infect Dis* 2005; 191:666-77; <http://dx.doi.org/10.1086/428405>

- [30] Altfield M, Rosenberg ES. The role of CD4(+) T helper cells in the cytotoxic T lymphocyte response to HIV-1. *Curr Opin Immunol* 2000; 12:375-80; PMID:10899028; [http://dx.doi.org/10.1016/S0952-7915\(00\)00103-5](http://dx.doi.org/10.1016/S0952-7915(00)00103-5)
- [31] Mann JK, Ndung'u T. HIV-1 vaccine immunogen design strategies. *Virology* 2015; 12:3; PMID:25616599; <http://dx.doi.org/10.1186/s12985-014-0221-0>
- [32] Jin X, Newman MJ, De-Rosa S, Cooper C, Thomas E, Keefer M, Fuchs J, Blattner W, Livingston BD, McKinney DM, et al. A novel HIV T helper epitope-based vaccine elicits cytokine-secreting HIV-specific CD4+ T cells in a Phase I clinical trial in HIV-uninfected adults. *Vaccine* 2009; 27:7080-6; PMID:19786145; <http://dx.doi.org/10.1016/j.vaccine.2009.09.060>
- [33] Walker LE, Vang L, Shen X, Livingston BD, Post P, Sette A, Godin CS, Newman MJ. Design and preclinical development of a recombinant protein and DNA plasmid mixed format vaccine to deliver HIV-derived T-lymphocyte epitopes. *Vaccine* 2009; 27:7087-95; PMID:19786132; <http://dx.doi.org/10.1016/j.vaccine.2009.09.059>
- [34] Wilson CC, McKinney D, Anders M, MaWhinney S, Forster J, Crimi C, Southwood S, Sette A, Chesnut R, Newman MJ, et al. Development of a DNA vaccine designed to induce cytotoxic T lymphocyte responses to multiple conserved epitopes in HIV-1. *J Immunol* 2003; 171:5611-23; PMID:14607970; <http://dx.doi.org/10.4049/jimmunol.171.10.5611>
- [35] Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusim K, Funkhouser R, Kuiken C, Haynes B, Letvin NL, Walker BD, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. *Nat Med* 2007; 13:100-6; <http://dx.doi.org/10.1038/nm1461>
- [36] Santra S, Muldoon M, Watson S, Buzby A, Balachandran H, Carlson KR, Mach L, Kong WP, McKee K, Yang ZY, et al. Breadth of cellular and humoral immune responses elicited in rhesus monkeys by multi-valent mosaic and consensus immunogens. *Virology* 2012; 428:121-7; PMID:22521913; <http://dx.doi.org/10.1016/j.virol.2012.03.012>
- [37] Ndhlovu ZM, Piechocka-Trocha A, Vine S, McMullen A, Koofhe-thile KC, Goulder PJ, Ndung'u T, Barouch DH, Walker BD. Mosaic HIV-1 Gag antigens can be processed and presented to human HIV-specific CD8+ T cells. *J Immunol* 2011; 186:6914-24; PMID:21576505; <http://dx.doi.org/10.4049/jimmunol.1004231>
- [38] Santra S, Liao HX, Zhang R, Muldoon M, Watson S, Fischer W, Theiler J, Szinger J, Balachandran H, Buzby A, et al. Mosaic vaccines elicit CD8+ T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys. *Nat Med* 2010; 16:324-8; <http://dx.doi.org/10.1038/nm.2108>
- [39] Barouch DH, O'Brien KL, Simmons NL, King SL, Abbink P, Maxfield LF, Sun YH, La Porte A, Riggs AM, Lynch DM, et al. Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. *Nat Medicine* 2010; 16:319-23; <http://dx.doi.org/10.1038/nm.2089>
- [40] Oyarzun P, Kobe B. Computer-aided design of T-cell epitope-based vaccines: addressing population coverage. *Int J Immunogenet* 2015; 42(5):313-21
- [41] Sette A, Sidney J. HLA supertypes and supermotifs: a functional perspective on HLA polymorphism. *Curr Opin Immunol* 1998; 10:478-82; PMID:9722926; [http://dx.doi.org/10.1016/S0952-7915\(98\)80124-6](http://dx.doi.org/10.1016/S0952-7915(98)80124-6)
- [42] Reche PA, Reinherz EL. PEPVAC: a web server for multi-epitope vaccine development based on the prediction of supertypic MHC ligands. *Nucleic Acids Res* 2005; 33:W138-42; PMID:15980443; <http://dx.doi.org/10.1093/nar/gki357>
- [43] Zhang GL, DeLuca DS, Keskin DB, Chitkushev L, Zlateva T, Lund O, Reinherz EL, Brusica V. MULTIPRED2: a computational system for large-scale identification of peptides predicted to bind to HLA supertypes and alleles. *J Immunol Methods* 2011; 374:53-61; PMID:21130094; <http://dx.doi.org/10.1016/j.jim.2010.11.009>
- [44] Toussaint NC, Kohlbacher O. OptiTope—a web server for the selection of an optimal set of peptides for epitope-based vaccines. *Nucleic Acids Res* 2009; 37:W617-22; PMID:19420066; <http://dx.doi.org/10.1093/nar/gkp293>
- [45] Molero-Abraham M, Lafuente EM, Flower DR, Reche PA. Selection of conserved epitopes from hepatitis C virus for pan-population stimulation of T-cell responses. *Clin Dev Immunol* 2013; 2013:601943; PMID:24348677; <http://dx.doi.org/10.1155/2013/601943>
- [46] Vider-Shalit T, Raffaelli S, Louzoun Y. Virus-epitope vaccine design: informatic matching the HLA-I polymorphism to the virus genome. *Mol Immunol* 2007; 44:1253-61; PMID:16930710; <http://dx.doi.org/10.1016/j.molimm.2006.06.003>
- [47] Buggert M, Norstrom MM, Czarnecki C, Tupin E, Luo M, Gyllenstein K, Sönnnerborg A, Lundegaard C, Lund O, Nielsen M, et al. Characterization of HIV-specific CD4+ T cell responses against peptides selected with broad population and pathogen coverage. *PLoS One* 2012; 7:e39874; PMID:22792193; <http://dx.doi.org/10.1371/journal.pone.0039874>
- [48] Toussaint NC, Maman Y, Kohlbacher O, Louzoun Y. Universal peptide vaccines - optimal peptide vaccine design based on viral sequence conservation. *Vaccine* 2011; 29:8745-53; PMID:21875632; <http://dx.doi.org/10.1016/j.vaccine.2011.07.132>
- [49] Antonets DV, Bazhan SI. PolyCTLDesigner: a computational tool for constructing polyepitope T-cell antigens. *BMC Res Notes* 2013; 6:407; PMID:24107711; <http://dx.doi.org/10.1186/1756-0500-6-407>