Available online at www.sciencedirect.com

ScienceDirect

Procedia in Vaccinology 1 (2009) 15–22

Procedia in Vaccinology

www.elsevier.com/locate/procedia

2nd Vaccine Global Congress, Boston 2008

Immunome-derived Epitope-driven Vaccines (ID-EDV) Protect against Viral or Bacterial Challenge in Humanized Mice

Lenny Moise[a,b], Matt Ardito[a], Joe Desrosiers[a], Jill Schriewer[c], Mark Buller[d], Sharon E. Frey[d], Stephen H. Gregory[b,e], Steven F. Moss[b,d], Jinhee Lee[e], Hardy Kornfeld[e], Bill Martin[a], **Anne S. De Groot[a,b,c,*]**

[a]*EpiVax, 146 Clifford Street, Providence, RI 02903, USA*
[b]*Alpert Medical School, Brown University, Providence, RI 02903, USA*
[c]*Institute for Immunology and Informatics, University of Rhode Island, 80 Washington Street, Providence, RI 02903, USA*
[d]*Saint Louis University, St. Louis, MO 63104, USA*
[e]*Lifespan, Rhode Island Hospital, Providence RI, 02903, USA*
[f]*University of Massachusetts Medical Center, Worcester, MA 01655, USA*

Abstract

While whole killed, whole protein, or live attenuated vaccines were the standard bearers for protective vaccines in the last century, there are concerns about their safety. New vaccine design techniques are contributing to an emphasis on vaccines developed using the minimum essential subset of T- and B-cell epitopes that comprise the "immunome." We have used bioinformatics sequence analysis tools, epitope-mapping tools, microarrays and high-throughput immunology assays to identify the minimal essential vaccine components for smallpox, tularemia, *Helicobacter pylori* and tuberculosis vaccines. As will be described in this review, this approach has resulted in the development of four immunome-derived epitope-driven vaccines (ID-EDV); three of these proved protective against viral or bacterial challenge. Protective efficacies of 100% (vaccinia), 90% (*H. pylori*), and 57% (tularemia) were achieved in HLA-transgenic (humanized) mouse models and the p27 knockout mouse (for *H. pylori*). Such immunome-derived vaccines have a significant advantage over conventional vaccines; the careful selection of the components should diminish undesired side effects such as those observed with whole pathogen and protein subunit vaccines. Here we summarize data showing prototype ID-ED vaccine protection against lethal challenge with vaccinia, tularemia, and *H. pylori* in a model of infection. The tools that made these successes possible are described and the anticipated clinical development of ID-ED vaccines is discussed.

© 2009 Elsevier B.V. All rights reserved

Keywords: Immunome; vaccine; T-cell; epitope; tularemia; smallpox: helicobacter

1877-282X/$–see front matter © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.provac.2009.07.004

1. Rationale for a new approach to vaccines

During the 20th Century, many classic childhood diseases were nearly eradicated in the developing world, but many pathogens and emerging infections still remain for which the original attenuation, inactivation, or subunit approaches to vaccine development did not work. As pathogens evolve, so must vaccine design. Immunome derived, also known as epitope-driven, vaccines represent the next phase of vaccine design. Instead of using whole pathogens or proteins, immunome-derived epitope-driven vaccines use the smallest functional unit of immunity, the epitope, to tailor an effective immune response [1,2].

One reason to develop immunome-derived epitope-driven vaccines (ID-EDV) is that the safety of live attenuated microorganisms or inactivated microorganisms administered through injections has been questioned. Adverse incidents have been linked to incomplete attenuation or inactivation of such "whole" bacterial or viral vaccines. Evidence is also emerging that cross-reactive epitopes may predispose the vaccinated subject to an altered immune response to vaccination, leading to an alteration in the protective effect of the vaccine [3,4]. Additionally, whole-killed or attenuated vaccines may contain proteins or other components (cell wall lipids, polysaccharides) that elicit an undesired immune response. Therefore, as researchers have become more knowledgeable about immune response targets and determinants, a more favorable strategy for vaccine development may be to select specific subunits, such as T-cell epitopes and B-cell epitopes from microorganisms, and produce them as recombinant vaccines.

Pre-exposure to certain bacteria or viruses may also determine the outcome of vaccination. Such cross-reactive immune responses, termed "heterologous immunity," have now been shown to alter the effectiveness of vaccines and therapeutics [5,6] in animal models. Cross-reactive immune responses may also alter immune response to self, leading to autoimmune disease, a health problem of steadily increasing proportions that now affects one in twenty Americans and Europeans. In-depth studies of autoimmunity have revealed HLA associations and a number of environmental triggers [7,8]. In some studies, cross-reactive T-cell responses have been attributed to epitopes present in certain vaccines [9]. The relationship between human pathogens, commensal microbes, and the (human) self at the T-cell epitope level may deserve more attention than it has received to date.

Critical to the development of ID-EDV has been the elucidation of the 'correlates of immunity' for a wide range of important human pathogens. The link between epitope-specific responses, establishment of T-cell memory, and protection from disease has been confirmed for HIV, hepatitis B (HBV), hepatitis C (HCV), malaria [10-12], and other infectious diseases. A number of laboratories are pursuing research supporting the hypothesis that a protective immune response to a number of pathogens requires the development of broad T-cell responses to an ensemble of different epitopes [13- 16]. Following exposure to a pathogen, epitope-specific memory T-cell clones are established [17]. These clones respond rapidly and efficiently upon subsequent infection, secreting cytokines, killing infected host cells, and marshalling other cellular defenses against the pathogen. Indeed, T cells have come to be recognized as critical mediators of competent and lasting humoral and cytotoxic immune responses elicited by vaccines [18,19]. In-silico epitope-mapping tools are often used to screen large numbers of protein sequences for potential T-cell epitopes. In high-throughput screening, small differences in the accuracy of epitope prediction tools can contribute to large differences in the cost and time-effort expended on subsequent studies.

The focus on T-cell epitopes for vaccine design catalyzed the development of computer-driven (immunoinformatics) methods for defining T-cell epitopes directly from protein sequences. This enabled investigations of the role of T-cells to leapfrog directly to the leading edge of immunology and vaccine research. For example, the research team of Martin and De Groot at EpiVax, Inc., has developed a suite of computer algorithms that can be applied to the development of epitope-based vaccines; this suite includes EpiMatrix, ClustiMer, Conservatrix, BlastiMer, Aggregatrix, OptiMatrix, and VaccineCAD [20- 28].

EpiMatrix was first developed in 1996 [29] and subsequently updated for use with Oracle databases by Bill Martin [30]. EpiMatrix was recently evaluated for its ability to predict established class I and class II HLA-restricted epitopes and these data were compared to an analysis published by Wang et al. [31] and the IEDB [32]. An "area under the Receiver Operated Characteristic (ROC) curve" was used to compare EpiMatrix to published data for six "supertype" class I and eight archetypal class II EpiMatrix matrices developed in 2008. The analyses were performed using published epitope data available in the IEDB as of June 2008 [33]. For Class I and Class II predictions, the average area under curve (AUC) values of EpiMatrix were .95 and .78, respectively. These values outperform the best tools available (IEDB ANN) as well as additional publicly available tools used in the

comparison (RANKPEP, BIMAS, SYFPEITHI, MHC2PRED, MHCPRED, SMM-align, SVRMHC, and TEPITOPE). The comparisons are now published [34].

In addition to the EpiMatrix algorithm for T-cell epitope identification, the EpiMatrix toolset also includes a set of analysis and design tools directly applicable to the vaccine design process. **ClustiMer,** an ancillary algorithm used with EpiMatrix, maps MHC motif matches along the length of a protein and calculates the density of motifs for eight common class II HLA alleles: DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, DRB1*1301 and DRB1*1501. Typical T-cell epitope 'clusters' range from 9 to roughly 25 amino acids in length and, considering their affinity to multiple alleles and across multiple frames, can contain anywhere from 4 to 40 binding motifs, also known as promiscuous epitopes.

The **Conservatrix** algorithm identifies conserved segments from among any given set of variable protein isolates. Pairing EpiMatrix with Conservatrix allows users to identify peptides, which are both potentially antigenic and conserved in circulating disease strains. **BlastiMer** compares a peptide's sequence to the human proteome to ensure that it does not contain too much homology to any human protein. The **Aggregatrix** algorithm addresses the classical "set cover" problem by guiding the selection of a portfolio of epitopes that collectively "cover" a wide variety of both the known circulating strain variants of a given pathogen and the majority of common human HLA types. **OptiMatrix** is an algorithm that is used for designing altered peptide ligands that optimize the "aggretope." Specifically, OptiMatrix guides strategic substitutions of the MHC-contact residues such that the peptide binds more strongly; the TCR-facing residues are free to interact as they would in the unaltered peptide. The **VaccineCAD** algorithm arranges putative T-cell epitopes to create optimized "string-of-beads" vaccine immunogens. The **EpiAssembler** algorithm was developed especially for use with highly variable RNA viruses. It is used to analyze the universe of viral isolates and to create composite epitope sequences whose constituent overlapping epitopes are both highly conserved and highly immunogenic [27]. Taken collectively, these tools allow researchers to quickly and effectively identify T-cell epitopes and design new antigens for experimental study, as illustrated in the next three sections.

EpiVax' immunoinformatics toolkit has been used to rank proteins for potential immunogenicity [35,36] and to design and evaluate vaccines [37,38]. We note that Korber et al. recently implemented very similar tools for the design of HIV-1 vaccines [39]. Over the course of a decade of research, the EpiVax tools have been validated *in vitro* and *in vivo*. The tools are currently in use for both vaccine and protein therapeutics design [40 -44]. In the next few paragraphs, we summarize data showing that prototype ID-ED vaccines, developed using the EpiVax vaccine design toolkit, provide protection against lethal challenge with vaccinia, tularemia, and in a model of chronic *H.pylori* infection. We describe the tools that made these successes possible and discuss the anticipated clinical development of ID-ED vaccines.

2. New immunome-derived, epitope-driven vaccines: protection against challenge

2.1. ID-EDV for F. tularensis (TuliVax)

Francisella tularensis causes the potentially fatal disease, tularemia, which is of great concern in regions of the world where tularemia is endemic and, in addition, because of the potential for its use in a bioterror attack. We employed the genome-to-vaccine strategy to develop a T-cell epitope-based tularemia vaccine. The EpiMatrix epitope-mapping algorithm was used to identify promiscuous T-cell epitopes within secreted proteins predicted from an analysis of the *F. tularensis* subsp. *tularensis* genome; T-cell epitopes within known expressed proteins were also identified. The top-scoring 40 putative promiscuous class II epitopes were screened *in vitro* using a recombinant soluble HLA class II competition binding assay. High affinity epitopes were tested in ELISpot assays using blood from human subjects that had recovered from *F. tularensis tularensis* infection. Soluble DRB1*0101 bound 42% of the predicted class II peptides with high affinity. ELISpot assays, performed using blood from tularemia infected patients, showed positive IFN-gamma responses to 21 of 25 individual Class II peptides and to peptide pools. Fourteen Class II epitope sequences that elicited a robust response in these two assays were incorporated into a DNA vaccine construct. DRB1*0101 transgenic mice were immunized with the multi-epitope construct, boosted with epitope peptides formulated in liposomes with CpG oligonucleotides, then challenged intratracheally with a lethal dose of the *F. tularensis* vaccine strain.

Splenocytes from mice vaccinated with the string-of-beads DNA vaccine construct, followed by boosting with peptides in liposomes (with CpG), showed a higher response to the vaccine peptides than did splenocytes from sham control mice. These responses correlated with protection: >50% of immunized mice survived respiratory challenge with 5xLD_{50} of live, attenuated *F. tularensis* subsp. *holarctica* (LVS) while all non-immunized mice died. This study demonstrated that T-cell epitopes predicted from the *F. tularensis* genome could protect against live aerosol challenge in a humanized small animal model [45]. An improved vaccine with additional *F. tularensis tularensis* epitopes is currently under evaluation in mice challenged with the more virulent SchuS4 strain of *F. tularensis*.

2.2. ID-EDV for Smallpox (VennVax)

To develop a novel, safer smallpox vaccine that could be used alone or in combination with existing attenuated vaccines (MVA, ACAM2000), we screened three variola genomes and four vaccinia genomes for conserved candidate epitopes using EpiMatrix, ClustiMer and Conservatrix. Our approach to this problem was as follows: 908,040 9-10mer peptides from 1,472 non-redundant genes were examined with our Conservatrix and EpiMatrix tools and 10,147 (6%) were found to be conserved in all strains and potentially immunogenic. We selected 110 of the conserved (Venn-diagramed, hence VennVax) epitopes for vaccine design, of which 50 were promiscuous Class II epitopes, 40 were Class I HLA A2 and 20 were Class I B7. Ninety-one percent of epitopes were confirmed in ELISpot assays using blood from Dryvax immunized volunteers. Epitopes were engineered into string-of-beads multi-epitope genes using VaccineCAD and subcloned into pVAX1. This prototype vaccine is called the VennVax smallpox vaccine as it incorporates epitopes at the intersection of the variola and vaccinia immunomes.

Initially, DRB1*0101 transgenic mice were immunized intramuscularly with the VennVax DNA construct and boosted subcutaneously with the corresponding peptides (in liposomes with CpG). In a follow up study, two groups of DRB1*0301 transgenic mice were intramuscularly immunized and boosted intranasally with peptides formulated in liposomes with CpG oligodeoxynucleotide. Immunogenicity was measured by IFNγ ELISpot. Variola/vaccinia epitopes that stimulated >20 SFC/10^6 splenocytes in comparison with non-immunized mice ($p<0.01$) were considered immunogenic. Immunization of DRB1*0101 transgenic mice stimulated significant T cell responses to 6 of 25 epitopes (24%). In comparison, DRB1*0301 mice immunized with the same 25-epitope set responded to 10 (40%) of epitopes, of which two were also reactive in DRB1*0101 mice. A vaccine encoding a second set of 25 epitopes stimulated significant responses for 8 (32%) epitopes in DRB1*0301 mice. In this study, more variola/vaccinia epitopes were confirmed for less cost and in less time than two previously published studies [46,47]. Immunization with the fifty epitope VennVax DNA vaccine followed by boosting with peptide-in-liposome provided 100% efficacy against aerosolized vaccinia challenge. Prior to challenge, antibodies to vaccinia were not detectable in the vaccinated mice. To our knowledge, the 100% protective VennVax vaccine is the only effective T cell epitope-based vaccine described for vaccinia, to date. The next phase of VennVax vaccine development will involve testing efficacy of the DNA prime with vaccinia boost in a monkeypox model.

2.3. ID-EDV for H. pylori (HelicoVax)

Using EpiMatrix, open reading frames (ORFs) that are highly conserved between *H. Pylori* human strains J99 and 26695 were analyzed for T-cell epitope clusters with motifs for HLA Class II and the mouse I-Ab alleles. Fifty epitopes were incorporated into "HelicoVax", a DNA-prime/peptide-boost vaccine. HelicoVax was tested in p27$^{-/-}$ C57BL/6 mice pre-infected with murine-*adapted* SS1 strain, a novel model for *H. pylrori*–associated gastric carcinogenesis [48]. Immunogenicity and modulation of immune response were measured 45 weeks post-immunization. We identified 1,152 epitope clusters from 1,107 conserved ORFs. From the top 150 epitopes, 50 were selected for vaccine formulation based on their high affinity for HLA DR1, defined by >50% inhibition of reference peptide at 10 mM in a competition assay. Interferon-gamma ELISpot assays of epitope-stimulated splenocytes demonstrated that 47/50 peptides (94%) were immunogenic following IN or IM DNA immunization with the multi-epitope vaccine as compared with only 4/50 epitopes recognized in SS1 lysate-immunized animals. Pathology results and culture results are pending. In addition, mice infected with *H. pylori* displayed reduced regulatory T cell populations (CD4+ CD25+ double positive cells) among both splenocytes (41% reduction, p=0.098) and mesenteric lymph node cells (35% reduction, p=0.026) compared to non-exposed mice. Similar reductions in numbers of Tregs were seen among mice immunized with SS1 lysate (splenocytes: 48% reduction

p=0.02; lymph node cells: 28% reduction p=0.036). This is an important observation as suppression of immune response is considered one of the factors that contribute to the carcinogenic properties of *H. pylori* infection. Additional studies are planned which will explore the development of gastric cancer in *H.pylori*-exposed, HelicoVax- immunized mice.

3. Conclusion

In the cancer vaccine field, where the concept of T-cell epitope-driven vaccines is well accepted, a number of epitope-driven vaccines have successfully passed preclinical tests and are either currently in Phase I/II clinical trials or trials are soon to be initiated. By contrast, the immunome-derived, epitope-driven vaccine approach has been less frequently pursued for microbial pathogens, and as a consequence, few such vaccines have reached the stage of Phase I or II efficacy trials in humans [49,50].

Indeed, banner headlines about the failure of the Merck HIV vaccine have dampened enthusiasm about vaccines that evoke T-cell epitope-driven immune responses for human pathogens [51]. To better understand the reason for the failure of that or other similar "T-cell directed" vaccines, it is important to remember that the breadth and the quality of the immune response play important roles in vaccine efficacy. In the context of HIV, better-designed immunogens (such as the cross-conserved epitopes identified for the GAIA Vaccine [27,52,53]) should improve the efficacy against 'wild type' HIV transmitted in human populations. Therefore, rather than abandon all hope based on the failure of the two best known "T-cell-directed" HIV vaccines [54,55], we and others believe that carefully designed epitope-driven vaccines will provide a successful means of vaccinating against HIV [56].

So as to improve on previous vaccines, future epitope-driven vaccines (against HIV and other human pathogens) should (1) expand on the number of epitopes contained in the vaccines, (2) pay careful attention to sequence conservation with clinical strains, (3) expand HLA population coverage, and (4) improve the vaccine delivery vehicle. Route of delivery also appears to be an important factor, particularly when the infection is by a mucosal route. These important factors that contribute to the success of a vaccine can be summarized as follows:

Immunogen + Adjuvant + Delivery vehicle = Vaccine.

One means of addressing immunogen quality is to include more epitopes. Evidence from animal studies suggests that the number of epitopes required for full protection is a small and definable subset [57,58]. Careful selection of promiscuous Class II epitopes and Class I supertype epitopes can provide greater than 99% coverage of human populations [59]. An effective ID-ED vaccine for humans may need to include at least 50 Class I and Class II broadly HLA-restricted (promiscuous) epitopes. Another means of improving the immunogenicity of an ID-EDV is to choose epitopes that induce multi-functional T cell responses in human PBMC [60]. Careful selection of vaccine delivery vehicle, route and formulation will improve immunogenicity and may also improve the likelihood of successful protection against disease.

The availability of higher-quality, validated vaccine design tools, such as the ones described here, enable the effective selection of highly immunogenic, cross-conserved T-cell epitopes. The delivery of these epitopes by the right route, in properly designed constructs and effective delivery vehicles, with the right adjuvant is likely to improve immunome-derived epitope-driven vaccine success and lead to important public health breakthroughs for future generations.

---

* *Anne S. De Groot, corresponding author. Tel.: +1-401-272-2123; fax: +1-401-272-7562.*
E-mail address: annied@epivax.com

1. Martin, W, Ardito M, Tassone R., Desrosiers J., Knopf, P. McMurry JA, Moise L., De Groot AS. Tools for Vaccine Design: Prediction and Validation of Highly Immunogenic and Conserved Class II Epitopes and Development of Epitope-driven Vaccines. For book entitled, Development of Vaccines: From Discovery to Clinical Testing. Editors Indrish Srivastava and Manmohan Singh Publisher: John Wiley and Sons.
2 De Groot, A. S., Bosma, A., Chinai, N., Frost, J., Jesdale, B. M., Gonzalez, B. M., Martin, W., and Saint-Aubin, C. From genome to vaccine: in silico predictions, ex vivo verification. Vaccine. 2001;19:4385-4395.
3. Mathurin KS, Martens GW, Kornfeld H, Welsh RM.CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. J Virol. 2009 Apr;83(8):3528-39

4. Chen HW, Zhang Z, Huber E, Chao CC, Wang H, Dasch GA, Ching WM. Identification of cross-reactive epitopes on the conserved 47 kDa antigen of Orientia tsutsugamushi and human serine protease. Infect Immun. 2009 Mar 16. (Epub).
5. Mathurin KS, Martens GW, Kornfeld H, Welsh RM. CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. J Virol. 2009 Apr;83(8):3528-39. Epub 2009 Feb 4
6. P Welsh RM, Fujinami RS. Pathogenic epitopes, heterologous immunity and vaccine design. Nat Rev Microbiol. 2007 Jul;5(7):555-63. Review.
7. Grant SF, Hakonarson H.Genome-wide association studies in type 1 diabetes. Curr Diab Rep. 2009 Apr;9(2):157-63.
8. Aarnisalo J, Veijola R, Vainionpää R, Simell O, Knip M, Ilonen J.Cytomegalovirus infection in early infancy: risk of induction and progression of autoimmunity associated with type 1 diabetes.Diabetologia. 2008 May;51(5):769-72. Epub 2008 Feb 16.
9. Markovic-Plese S, Hemmer B, Zhao Y, Simon R, Pinilla C, Martin R. High level of cross-reactivity in influenza virus hemagglutinin-specific CD4+ T-cell response: implications for the initiation of autoimmune response in multiple sclerosis. J Neuroimmunol. 2005 Dec;169(1-2):31-8. Epub 2005 Sep 15.
10. Blattman JN, Sourdive DJ, Murali-Krishna K, Ahmed R, Altman JD. Evolution of the T cell repertoire during primary, memory, and recall responses to viral infection. J Immunol. 2000 Dec 1;165(11):6081-90.
11. Harrer T, Harrer E, Kalams SA, Barbosa P, Trocha A, Johnson RP, Elbeik T, Feinberg MB, Buchbinder SP, Walker BD. Cytotoxic T lymphocytes in asymptomatic long-term nonprogressing HIV-1 infection. Breadth and specificity of the response and relation to in vivo viral quasispecies in a person with prolonged infection and low viral load. J Immunol. 1996 Apr 1;156(7):2616-23.
12. Doolan DL, Wizel B, Hoffman SL. Class I HLA-restricted cytotoxic T lymphocyte responses against malaria--elucidation on the basis of HLA peptide binding motifs. Immunol Res. 1996;15(4):280-305. Review.
13. Gianfrani, C., Oseroff, C., Sidney, J., Chesnut, R.W., Sette, A. Human memory CTL response specific for influenza A virus is broad and multispecific Hum Immunol. 2000;61:438-452.
14. Gillespie GM, Wills MR, Appay V, O'Callaghan C, Murphy M, Smith N, et al. Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors J Virol. 2000;74:8140-8150.
15 Kong WP, Wu L, Wallstrom TC, Fischer W, Yang ZY, Ko SY, Letvin NL, Haynes BF, Hahn BH, Korber B, Nabel GJ.Expanded breadth of the T-cell response to mosaic human immunodeficiency virus type 1 envelope DNA vaccination.J Virol. 2009 Mar;83(5):2201-15. Epub 2008 Dec 24.
16 Ramduth D, Day CL, Thobakgale CF, Mkhwanazi NP, de Pierres C, Reddy S, van der Stok M, Mncube Z, Nair K, Moodley ES, Kaufmann DE, Streeck H, Coovadia HM, Kiepiela P, Goulder PJ, Walker BD. Immunodominant HIV-1 Cd4+ T cell epitopes in chronic untreated clade C HIV-1 infection. PLoS ONE. 2009;4(4):e5013. Epub 2009 Apr 7.
17. Blattman JN, Sourdive DJ, Murali-Krishna K, Ahmed R, Altman JD. Evolution of the T cell repertoire during primary, memory, and recall responses to viral infection. J Immunol. 2000;165(11):6081-6090.
18. Gillespie GM, Wills MR, Appay V, O'Callaghan C, Murphy M, Smith N, Sissons P, Rowland-Jones S, Bell JI, Moss PA. Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. J Virol. 2000 Sep;74(17):8140-50.
19. Gianfrani C, Oseroff C, Sidney J, Chesnut RW, Sette A Human memory CTL response specific for influenza A virus is broad and multispecific. Hum Immunol. 2000 May;61(5):438-52.
20. Ahlers JD, Belyakov IM, Thomas EK, Berzofsky JA. High-affinity T helper epitope induces complementary helper and APC polarization, increased CTL, and protection against viral infection. J Clin Invest. 2001;108:1677-85.
21. De Groot AS, Sbai H, Saint-Aubin C, McMurry JA, Martin W. Immuno-informatics: mining genomes for vaccine components. Immunol. Cell. Biol. 2002;80:255–269.
22. Inaba H, Martin W, De Groot AS, Qin S, De Groot LJ. Thyrotropin receptor epitopes and their relation to histocompatibility leukocyte antigen-DR molecules in Graves' disease. J Clin Endocrinol Metab. 91(2006) 2286-94.
23. De Groot AS, Jesdale BM, Szu E, Schafer JR, Chicz RM, Deocampo G. An interactive Web site providing major histocompatibility ligand predictions: application to HIV research. AIDS Res Hum Retroviruses. 1997;13:529-31.

24. Bond KB, Sriwanthana B, Hodge TW, De Groot AS, Mastro TD, Young NL, Promadej N, Altman JD, Limpakarnjanarat K, McNicholl JM. An HLA-directed molecular and bioinformatics approach identifies new HLA-A11 HIV-1 subtype E cytotoxic T lymphocyte epitopes in HIV-1-infected Thais. AIDS Res Hum Retroviruses. 2001;20;703-17.
25. McMurry J, Sbai H, Gennaro ML, Carter EJ, Martin W, De Groot AS. Analyzing Mycobacterium tuberculosis proteomes for candidate vaccine epitopes. Tuberculosis (Edinb). 2005;85:95-105.
26. Dong Y, Demaria S, Sun X, Santori FR, Jesdale BM, De Groot AS, Rom WN, Bushkin Y. HLA-A2-restricted CD8+-cytotoxic- T cell responses to novel epitopes in Mycobacterium tuberculosis superoxide dismutase, alanine dehydrogenase, and glutamine synthetase. Infect Immun. 2004;72:2412-5.
27. Koita OA, Dabitao D, Mahamadou I, Tall M, Dao S, Tounkara A, Guiteye H, Noumsi C, Thiero O, Kone M, Rivera D, McMurry JA, Martin W, De Groot AS. Confirmation of immunogenic consensus sequence HIV-1 T cell epitopes in Bamako, Mali and Providence, Rhode Island. Hum Vaccin. 2006;2(3):119-28.
28. De Groot AS. Immunomics: Discovering New Targets for Vaccine and Therapeutics. Drug Discovery Today. 2006;11:203-9.
29. Jesdale BM, Deocampo G, Meisell J, Mullen L, Beall J, Marinello M, A.S. De Groot, Matrix-based prediction of MHC-binding peptides: the Epimatrix algorithm, reagent for HIV research, Vaccines 97, pp. 57-64, Cold Spring Harbor Laboratory Press 1997.
30. De Groot A.S., Bosma A, Chinai N, Frost J, Jesdale BM, Gonzalez MA, Martin W., Saint-Aubin C. From genome to vaccine: In silico predictions, ex vivo verification. Vaccine 2001 Aug 14; 19(31):4385-95.
31. Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol. 2008 Apr 4;4(4):e1000048.
32. Peters B, Sidney J, Bourne P, Bui HH, Buus S, Doh G, Fleri W, Kronenberg M, Kubo R, Lund O, Nemazee D, Ponomarenko JV, Sathiamurthy M, S choenberger S, Stewart S, Surko P, Way S, Wilson S, Sette A. The immune epitope database and analysis resource: from vision to blueprint. PLoS Biol. 2005 Mar;3(3):e91.
33. Zhang Q, Wang P, Kim Y, Haste-Andersen P, Beaver J, Bourne PE, Bui HH, Buus S, Frankild S, Greenbaum J, Lund O, Lundegaard C, Nielsen M, Ponomarenko J, Sette A, Zhu Z, Peters B. Immune epitope database analysis resource (IEDB-AR). Nucleic Acids Res. 2008 Jul 1;36(Web Server issue):W513-8.
34. De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol. 2009 May;131(2):189-201
35. Koren E, De Groot AS, Jawa V, et al (2007) Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein. Clin Immunol 124(1):26-32
36. De Groot AS, Moise L, (2007) Prediction of immunogenicity for therapeutic proteins: state of the art. Curr Opin Drug Discov Devel 10(3):332-340
37. De Groot AS, Scott DW (2007) Immunogenicity of protein therapeutics. Trends Immunol 28(11):482-490.
38. De Groot A.S., P. M. Knopf, D. Rivera and W. Martin. Immunoinformatics applied to modifying and improving biological therapeutics. Kluwer publications *Immunoinformatics*. Series: Immunomics Reviews: Vol. 1. Schönbach, Christian; Ranganathan, Shoba; Brusic, Vladimir (Eds.) 2007, ISBN: 978-0-387-72967-1. Available: September 21, 2007.
39. Thurmond J, Yoon H, Kuiken C, et al (2008) Web-based design and evaluation of T-cell vaccine candidates. Bioinformatics 24(14):1639-1640
40. De Groot AS, Jesdale BM, Szu E, et al (1997) An interactive Web site providing major histocompatibility ligand predictions: application to HIV research. AIDS Res Hum Retroviruses 13:529-531
41. Bond KB, Sriwanthana B, Hodge TW, et al (2001) An HLA-directed molecular and bioinformatics approach identifies new HLA-A11 HIV-1 subtype E cytotoxic T lymphocyte epitopes in HIV-1-infected Thais. AIDS Res Hum Retroviruses 20:703-717
42. McMurry J, Sbai H, Gennaro ML, et al (2005) Analyzing Mycobacterium tuberculosis proteomes for candidate vaccine epitopes. Tuberculosis 85:95-105
43. Dong Y, Demaria S, Sun X, et al (2004) HLA-A2-restricted CD8+-cytotoxic- T cell responses to novel epitopes in Mycobacterium tuberculosis superoxide dismutase, alanine dehydrogenase, and glutamine synthetase. Infect Immun 72: 2412-2415
44. Tatarewicz SM, Wei X, Gupta S, et al (2007) Development of a maturing T-cell-mediated immune response in patients with idiopathic Parkinson's disease receiving r-metHuGDNF via continuous intraputaminal

infusion. J Clin Immunol 27(6):620-627
45. McMurry JA, Moise L, Gregory SH, De Groot AS. Tularemia vaccines - an overview. Med Health R I. 2007 Oct;90(10):311-4.
46. Smith CL, Mirza F, Pasquetto V, Tscharke DC, Palmowski MJ, Dunbar PR, Sette A, Harris AL, Cerundolo V Immunodominance of poxviral-specific CTL in a human trial of recombinant-modified vaccinia Ankara. J Immunol. 2005 Dec 15;175(12):8431-7.
47. Jing L, Davies DH, Chong TM, Chun S, McClurkan CL, Huang J, Story BT, Molina DM, Hirst S, Felgner PL, Koelle DM. An extremely diverse CD4 response to vaccinia virus in humans is revealed by proteome-wide T-cell profiling. J Virol. 2008 Jul;82(14):7120-34. Epub 2008 May 14. Erratum in: J Virol. 2009 Mar;83(5):2404.
48. Kuzushita N, Rogers AB, Monti NA, Whary MT, Park MJ, Aswad BI, Shirin H, Koff A, Eguchi H, Moss SF. p27kip1 deficiency confers susceptibility to gastric carcinogenesis in Helicobacter pylori-infected mice. Gastroenterology. 2005 Nov;129(5):1544-56.
49. Eliott SL, Suhrbier A, Miles JJ, Lawrence G, Pye SJ, Le TT, Rosenstengel A, Nguyen T, Allworth A, Burrows SR, Cox J, Pye D, Moss DJ, Bharadwaj M. Phase I trial of a CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. J Virol. 2008 Feb;82(3):1448-57. Epub 2007 Nov 21.
50. McKinney DM, Skvoretz R, Livingston BD, Wilson CC, Anders M, Chesnut RW, Sette A, Essex M, Novitsky V, Newman MJ. Recognition of variant HIV-1 epitopes from diverse viral subtypes by vaccine-induced CTL.J Immunol. 2004 Aug 1;173(3):1941-50.
51. Wilson CC, Newman MJ, Livingston BD, MaWhinney S, Forster JE, Scott J, Schooley RT, Benson CA. Clinical phase 1 testing of the safety and immunogenicity of an epitope-based DNA vaccine in human immunodeficiency virus type 1-infected subjects receiving highly active antiretroviral therapy. Clin Vaccine Immunol. 2008 Jun;15(6):986-94.
52. De Groot AS, Rivera DA, McMurry JA, S. Buus, Martin W., Identification of Immunogenic HLA-B7 “Achilles’ heel” Epitopes Within Highly Conserved Regions of HIV. Vaccine, 2008 26, 3059—3071.
53. De Groot AS, McMurry JA, Moise L, Martin W. Epitope-based Immunome-derived Vaccines: A Strategy for Improved Design and Safety. Springer Immunomics Series. Editor Andras Falus. Falus, Andras (Ed.) 2009.
54. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, Defawe OD, Carter DK, Hural J, Akondy R, Buchbinder SP, Robertson MN, Mehrotra DV, Self SG, Corey L, Shiver JW, Casimiro DR; Step Study Protocol Team. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis.Lancet. 2008 Nov 29;372(9653):1894-905. Epub 2008 Nov 13.
55. Wilson CC, Newman MJ, Livingston BD, MaWhinney S, Forster JE, Scott J, Schooley RT, Benson CA Clinical phase 1 testing of the safety and immunogenicity of an epitope-based DNA vaccine in human immunodeficiency virus type 1-infected subjects receiving highly active antiretroviral therapy. Clin Vaccine Immunol. 2008 Jun;15(6):986-94. Epub 2008 Apr 9.
56. Hanke T. STEP trial and HIV-1 vaccines inducing T-cell responses.Expert Rev Vaccines. 2008 Apr;7(3):303-9.
57. Moutaftsi M, Peters B, Pasquetto V, Tscharke DC, Sidney J, Bui HH, Grey H, Sette A. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. Nat Biotechnol. 2006 Jul;24(7):817-9.
58. Moise L, De Groot AS. Putting immunoinformatics to the test. Nat Biotechnol. 2006 Jul;24(7):791-2.
59. Sette A, Sidney J. Nine major HLA Class I supertypes account for the vast preponderance of HLA-A and –B polymorphism. Immunogenetics. 1999 Nov;50(3-4):201-12.
60. Wille-Reece U, Wu CY, Flynn BJ, Kedl RM, Seder RA. Immunization with HIV-1 Gag protein conjugated to a TLR7/8 agonist results in the generation of HIV-1 Gag-specific Th1 and CD8+ T cell responses. J Immunol. 2005 Jun 15;174(12):7676-83.