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Review Article

Vaccination for malaria based on genomics and proteomics

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CONTENTS

Abstract

The field of vaccinology has benefited from new bioinformatics technologies. The complete genome sequences of malaria parasites have been deciphered and vaccines can now be targeted towards specific gene products that traditional vaccine research failed to discover. Advanced technologies for vaccine development, such as genome sequence analysis, microarrays, proteomics approaches, high-throughput cloning and bioinformatics database tools, and computational vaccinology, can be applied to the development of vaccines for several diseases, including malaria. New advances in malaria genomics, proteomics and immunomics should also contribute to successful malaria vaccine development.

Introduction

Overview of vaccine research

Vaccines have been known and used for about two centuries. The present focus is on development trends and potential rather than individual vaccines. While the first vaccines were a result of scientific observation, subsequent development has been highly dependent on advances in microbiology to provide both the knowledge basis and the technology for new vaccines (1). Although vaccines exist against almost 30 different diseases, many of the available vaccines are far from ideal and the search continues for new vaccines (2). Infectious diseases account for 20% of global mortality, especially in children under the age of 5 years, and vaccines are effective at controlling these diseases, as demonstrated by the successful eradication of smallpox, the impressive progress towards the eradication of polio and the significant achievements in terms of measles-related mortality (3). Today, vaccines being used by the World Health Organization's (WHO's) Expanded Program on Immunization (EPI) against common childhood diseases such as diphtheria, whooping cough, tetanus, measles and tuberculosis reach 80% of the world's children and save an estimated millions of lives each year (4).

However, we need to construct a new immunization paradigm involving countries, industry, research institutions, foundations and international agencies like the WHO and the United Nations Children's Fund (UNICEF). Over the past decades, intensive research has focused on developing vaccine therapies for several infectious diseases. New safe and effective vaccines must be developed for a variety of infections of public health importance for which no effective preventive intervention measures are available (3). A number of substantial unresolved questions cloud the current approach, and the development of vaccines against these organisms has proved very challenging (4). Biotechnologists and vaccinologists are facilitating the rapid expansion of the search for new drugs, endowing clinicians with a better understanding of both diseases and novel alternatives for treating patients. Important research areas and tools include genomics, proteomics, ligand-receptor interactions, signal transduction, rational drug design, biochips and microarrays (5). Many candidate vaccines have been tested in animal models. The immunogenicity and safety of several vaccine formulations have been tested through clinical trials, but the efficacy of these vaccine therapies in humans must be determined in the near future (4).

How can genomics complement traditional approaches?

Vaccinology is a combinatorial science that involves the study of the diversity of pathogens and the human immune system, and formulations that can modulate immune responses and prevent or cure diseases (6). The conventional approach to vaccine development is based on dissection of the pathogen using biochemical, immunological and microbiological methods. Although

successful in several cases, this approach has failed to provide clues to prevent a number of infectious diseases (7).

Genomics projects have brought major advances in medical science. Vaccine research entered a new era when the complete genome of a pathogenic bacterium was published in 1995 (8). Genomics and proteomics projects and large-scale screening of pathogen-host and antigen-host interactions have also provided data (6). The complete genome sequences of more than 60 microorganisms have been elucidated in the past decade. Concurrently, a series of new informatics tools designed to harness this new wealth of information have been developed. Some of these new tools allow scientists to select regions of genomes that trigger immune responses (9). As for other fields of medical sciences, it is expected that vaccinology will benefit greatly from the emerging genomics technologies, such as bioinformatics, proteomics and DNA microarrays (10). The post-genomic era just starting therefore promises an exponential increase in vaccine research and new vaccines, both improved vaccines with greater efficacy and reduced adverse effects to replace old vaccines, and vaccines for the prevention of diseases for which none is currently available (1).

The availability of complete genome sequences together with novel advanced technologies has revolutionized the approach to vaccine development (7). Current developments in computational vaccinology mainly support the analysis of antigen processing and presentation and the characterization of targets of the immune response (6). At present, vaccine technologists are using microarrays, immunoinformatics, proteomics and high-throughput immunology assays to reduce the massive volume of information available in genome databases to a manageable size (8). Immunomics is a new science that addresses the interface between the host and pathogen proteome, bridging informatics, genomics, proteomics, immunology and clinical medicine (11). This large-scale screening of immune processes using powerful immunoinformatic tools offers great promise for the future translation of basic immunology research advances to successful vaccines (6).

Advanced technologies in vaccine development

Genome sequence analysis

The availability of the first complete microbial genome sequence in 1995 began a genomic era that has allowed medical scientists to change the paradigm and approach vaccine development based on genomic information (12). The availability of complete genomes is expected to provide a major contribution to vaccine development, particularly for targeting those pathogens for which traditional approaches have so far failed (13). Combining pathogen genome sequences with the host and vector genome sequences promises to be a robust method for the identification of host-pathogen interactions. In addition, comparative sequencing of related species, especially of organisms used as model systems in the study of the disease, is beginning to realize its potential in the identification of genes that are involved in evasion of the host immune response (7).

The genomics revolution allows the design of vaccines starting from the prediction of all antigens *in silico*, independently of their abundance and without the need to grow the pathogen *in vitro* (7). A process named “reverse vaccinology” is one of the most advanced technologies in vaccine development. This new genome-based approach has been successfully applied to *Neisseria meningitidis* serogroup B, for which conventional strategies have failed to provide an effective vaccine (7, 14, 15). The concept of reverse vaccinology can be easily applied to all pathogens for which vaccines are not yet available and can be extended to parasites and viruses (7).

Microarrays

The identification of an immune response correlate for protection against a pathogen would greatly facilitate the rational development of an effective vaccine. However, finding such a correlate has been a daunting task. DNA microarray technology is a new and powerful tool that allows the simultaneous analysis of a large number of nucleic acid hybridization experiments in a rapid and efficient fashion. The development of the DNA microarray chip has been driven by modern techniques of microelectronic manufacture, miniaturization and integration to produce what is referred to as “laboratory-on-chip” devices. An advantage of microarray technology is that it can assist researchers in better defining and understanding the expression profile of a given genotype associated with disease, adverse effects from exposure to certain stimuli, or in understanding or predicting immune responses to specific antigens (16). The development of DNA microarray technology a decade ago enabled the establishment of functional genomics as one of the most active and successful scientific disciplines. With the ongoing development of immunomic microarray technology — a spatially addressable, large-scale technology for the measurement of specific immunological responses— the new challenge of functional immunomics is emerging. Immunomic data have been successfully used to identify biological markers involved in several diseases and responses to vaccines (17).

Proteomics approach and high-throughput cloning

Despite the increasing availability of genome sequences for many human pathogens, the production of complete proteomes remains at a bottleneck. Traditionally, the production of a recombinant protein requires a preliminary cloning of the target gene into an expression vector before evaluating its cellular expression (18, 19). Among current methods, site-specific recombination cloning techniques, which eliminate the use of restriction endonucleases and ligases, offer sever-

al advantages in the context of high-throughput procedures (19). Rapid and highly efficient, the recombinational cloning technology is largely used for structural genomics and functional proteomics (18, 19). High-throughput approaches for gene cloning and expression require the development of new, nonstandard tools for use by molecular biologists and biochemists (20). A high-throughput PCR recombination cloning and expression platform has been developed that allows hundreds of genes to be batch-processed by using basic laboratory procedures (21). Expression library immunization (ELI) is a potent technology for discovering new vaccines and also for generating genomic vaccines with amplified, multivalent immunostimulatory capacities. ELI is accepted as a high-throughput technology to discover vaccine candidate genes by using the immune system to screen the entire genome of a pathogen for a vaccine candidate. At present, ELI has been instrumental in the discovery of new vaccine candidates from a number of different bacterial, fungal and parasitic pathogens. In addition, the process of applying ELI to the genome of pathogens allows us to genetically re-engineer these antigens to convert immunoevasive pathogen proteins to immunostimulatory vaccine antigens (18).

Bioinformatics database tools and computational vaccinology

Several bioinfomatics database tools are now available for vaccine development. Huge amounts of data are produced by genomics and proteomics projects and large-scale screening of pathogen-host and antigen-host interactions. For vaccine discovery, one can “mine” the genomic sequence for potential surface targets using various algorithms, characterize these gene targets and produce primers for cloning, all before entering the laboratory (22). Complete genome data for infectious microorganisms permit systematic computational sequence-based predictions and experimental testing of candidate vaccine epitopes. Both predictions and the interpretation of experiments rely on existing information in the literature, which is mostly manually extracted and analyzed (23). The heart of immunology is molecular recognition events that are indistinguishable from other types of biomacromolecular interactions. These can be addressed by quantitative experimental and theoretical biophysical techniques, and particularly by methods from drug design (24). Support of vaccine development through text-mining therefore requires the timely development of domain-specific extraction rules for full-text articles, and a knowledge model for epitope-related information (23).

Computational immunovaccinology is a new technique in vaccine development (24). Current developments in computational vaccinology mainly support the analysis of antigen processing and presentation and the characterization of targets of the immune response (25). The goal of immunoinformatics is to develop computational vaccinology as a potent tool in the quest for new vaccines (26). Databases and data-mining are the two principal weapons at the disposal of the *in silico* vaccinologist (Table I). Future development will also include systematic models of vaccine responses (25).

Advances in malaria genomics, proteomics and immunomics

Advances in malaria genomics

Based on recent advances in molecular biology, the genome project for malaria was launched (32-34). Of several species responsible for malaria, *Plasmodium falciparum* is the most serious and problematic and has been studied extensively. The *P. falciparum* genome project was founded in 1996 by an international consortium, with

Table I: Examples of bioinformatics database tools for vaccine development.

Database tool	Description
JenPep (27)	JenPep is a family of relational databases supporting the growing community of immunoinformaticians. It contains quantitative data on peptide binding to major histocompatibility complexes (MHCs) and transmembrane peptide transporters (TAPs), as well as an annotated list of T-cell epitopes.
MHCPred (28)	This is a server on the World Wide Web, a partial least squares-based multivariate statistical approach to the quantitative prediction of peptide binding to MHCs, the key checkpoint on the antigen presentation pathway within adaptive cellular immunity. MHCPred implements robust statistical models for both class I alleles (HLA-A*0101, HLA-A*0201, HLA-A*0202, HLA-A*0203, HLA-A*0206, HLA-A*0301, HLA-A*1101, HLA-A*3301, HLA-A*6801, HLA-A*6802 and HLA-B*3501) and class II alleles (HLA-DRB*0401, HLA-DRB*0401 and HLA-DRB*0701).
EPIMHC (29)	EPIMHC is a relational database of MHC-binding peptides and T-cell epitopes observed in real proteins. Currently, the database contains 4,867 distinct peptide sequences from various sources, including 84 tumor-associated antigens. Peptides resulting from a query can be selected to derive specific motif-matrices. Subsequently, these motif-matrices can be used in combination with a dynamic algorithm for predicting MHC-binding peptides from user-provided protein queries.
NERVE (30)	NERVE is a software environment for the *in silico* identification of the best vaccine candidates from whole proteomes of bacterial pathogens. The software integrates multiple robust and well-known algorithms for protein analysis and comparison. Vaccine candidates are ranked and presented in an HTML table showing relevant information and links to corresponding primary data.
PepDist (31)	This is a new framework for protein-peptide binding prediction based on learning peptide distance functions. This webserver provides binding predictions of peptides to 35 different MHC class I alleles.

support from private and government agencies in both the U.K. and the U.S. (33), with the idea that the success of the genome project would ultimately be determined by how rapidly and effectively the information it produced was utilized by the research community to advance the understanding of malaria. This project resulted in the Plasmodium Genome Database (PlasmoDB) (http://www.plasmodb.org). This database integrates sequence information, automated analyses and annotation data emerging from the *P. falciparum* genome sequencing consortium (32). Data in PlasmoDB are organized by chromosome (1-14), and can be accessed using a variety of tools for graphical and text-based browsing, or downloaded in various file formats (32, 34). There would be great benefit in integrating genomic sequence and functional genomics results with the large amount of pre-existing knowledge related to parasite biology and immunological interactions with the host. Attempts to achieve this include the PlasmoDB database, and the lessons learned in this effort could be of great utility to other organism-specific databases (35). The malaria parasite genome project has revealed certain metabolic pathways that can be targeted to develop antimalarial drugs and a large number of potential antigens for future potential vaccines (36, 37).

Advances in malaria proteomics

The recent completion of human, mosquito vector and parasite genomes relevant to the study of human malaria allows the application of modern proteomics technologies to complement previously implemented conventional approaches. Proteomic analysis has been employed to elucidate global protein expression profiles, the subcellular localization of gene products and host-pathogen interactions that are central to disease pathogenesis and treatment (38). Applied to *Plasmodium*, proteomics combines high-resolution protein or peptide separation with mass spectrometry and computer software to rapidly identify large numbers of proteins expressed during various stages of parasite development. Recent high-throughput proteomics approaches have provided a great amount of protein expression data on malaria, while smaller scale studies examining specific drug-related hypotheses are also being conducted (39).

Advances in malaria immunomics

Immunomics encompasses genomics, high-throughput and bioinformatics approaches to immunology (40). New advances in immunomics include specialty immunology databases, immunology database tools, immunome epitope research, epitope analysis tools, high-throughput technologies (gene sequencing, microarrays, proteomics), mathematical and theoretical models, and prediction tools (40, 41). Since an improved understanding of the human immune system and the genetic make-up of pathogens, together with advances in instrumentation and bioinformatics, have provided new insights into the variability of immune responses to vaccines within the human population, immunomics is currently at the center of vaccinology (42).

Malaria vaccination based on genomics and proteomics

Analysis of malaria genome sequences

Analysis of the malaria genome sequences has provided promising new leads for drug and vaccine development (43). Identification of the targets of protective T-cell or antibody responses from genomic data is at the heart of genome sequence analysis. However, the identification of antigens that will stimulate the most effective immunity against the target pathogen is still problematic since the malaria genome is large. The 23-Mb *P. falciparum* genome encodes more than 5,300 proteins, each of which is a potential target of protective immune responses (44).

Comparative genomics techniques are useful for determining differences among genes encoding vaccine candidate antigens. Recently, Safitri *et al.* studied the amino-terminal region of the serine repeat antigen (SERA) of *P. falciparum* as a major malaria vaccine candidate. They investigated the patterns of sequence diversity in exon II of the *SERA* gene and found that sequence variation in exon II might represent one of the parasite's strategies for immune evasion (45). Ferriera *et al.* analyzed sequence variations in block 2 repeats and in non-repetitive block 17, as well as other polymorphisms within the merozoite surface protein-1 (*MSP-1*) gene, in clinical isolates of *P. falciparum* (46). The merozoite surface protein of *Plasmodium* spp., which exhibits antigenic diversity among isolates, has been considered a vaccine candidate (47). The results indicated a role for nonhomologous recombination, such as strand-slippage mispairing during mitosis and gene conversion, in creating variation in a malaria antigen under strong diversifying selection (46).

Study of the malaria transcriptome

The complete annotated genome of the *P. falciparum* parasite is now available, thus providing a prediction of the possible gene products. This makes the application of functional genomics to malaria research feasible, with the final goal of providing a complete analysis of the malaria life cycle. Genome-wide approaches to the study of the transcriptome or proteome were successfully applied to the malaria parasite with the promise for vaccine candidates (48). Studies of gene expression in *Plasmodium* have evaluated each stage of malaria, from the pre-erythrocytic to the asexual and sexual erythrocytic stages. Kappe *et al.* analyzed the transcriptome of the malaria sporozoite stage. They identified expressed sequence tags (ESTs) for three proteins that may be involved in host cell invasion and documented their expression in sporozoites, facilitating the understanding of the pre-ery-

throcytic *Plasmodium* life cycle stages and the development of pre-erythrocytic vaccines (49). In another study, Young *et al.* performed a microarray analysis using ontology-based pattern identification to study the *P. falciparum* sexual development transcriptome. This analysis resulted in the identification of a sexual development cluster containing 246 genes, of which approximately 75% were hypothetical, exhibiting highly correlated, gametocyte-specific expression patterns. Inspection of the upstream promoter regions of the identified genes revealed putative *cis*-regulatory elements for sexual development transcriptional control mechanisms (50).

Study of the malaria proteome

The 23-Mb *P. falciparum* genome encodes more than 5,300 proteins, each of which is a potential target of protective immune responses. However, the current generation of subunit vaccines is based only on a single or a few antigens and therefore may elicit too narrow a response (44). Proteomics and computational analysis of these databanks are being used to model and investigate the three-dimensional structure of many key malaria proteins in an attempt to facilitate vaccine design. Recombinant protein expression in bacteria and yeast coupled with cGMP purification technologies and conditions have led to the identification of several dozen malaria protein antigens for phase I and vaccine trials (51). As an example of applied studies on the malaria proteome, Haddad *et al.* recently rapidly tested hundreds of DNA vaccines encoding exons from the *Plasmodium yoelii yoelii* genomic sequence. Orthologues of protective *P. yoelii yoelii* genes were then identified in the genomic databases of *P. falciparum* and *Plasmodium vivax* and investigated as candidate antigens for a human vaccine. Identified exons were then cloned into a DNA immunization vector with the Gateway cloning technology. High-throughput cloning of exons into DNA vaccines and their screening proved feasible and could rapidly identify new malaria vaccine candidate antigens (52). In another study, Aguiar *et al.* examined the feasibility of a high-throughput cloning approach using the Gateway system to create a large set of expression clones encoding *P. falciparum* single-exon genes. In this work, master clones and their open reading frames (ORFs) were transferred to multiple expression vectors and target genes were selected using specific sets of criteria, including stage expression and secondary structure, and the genes were subcloned into a DNA vaccine vector. In animal models, the functional expression of genes to generate antibodies against various stages of the parasite was observed (53).

In silico malaria vaccine design

Due to the advances in bioinformatics and chemoinformatics, *in silico* vaccine design is now feasible. Several bioinformatics and chemoinformatics tools can be applied to malaria vaccine development. Starting from the whole *Plasmodium* genome sequence, proteins regarded as potential vaccine candidates can be predicted, as presented in Figure 1. Potential vaccine candidates can be further processed by proteomics and computational analysis, as previously described.

References

1. Makela, P.H. *Vaccines, coming of age after 200 years.* FEMS Microbiol Rev 2000, 24: 9-20.

2. Combinations, the key to global immunization. CVI Forum 1993, (5): 2-6.

3. Kieny, M.P., Girard, M.P. *Human vaccine research and development: An overview.* Vaccine 2005, 23: 5705-7.

4. Alsahli, M., Farrell, R.J., Michetti, P. *Vaccines: An ongoing promise?* Dig Dis 2001, 19: 148-57.

5. Avidor, Y., Mabjeesh, N.J., Matzkin, H. *Biotechnology and drug discovery: From bench to bedside.* South Med J 2003, 96: 1174-86.

6. Brusic, V., August, J.T., Petrovsky, N. *Information technologies for vaccine research.* Expert Rev Vaccines 2005, 4: 407-17.

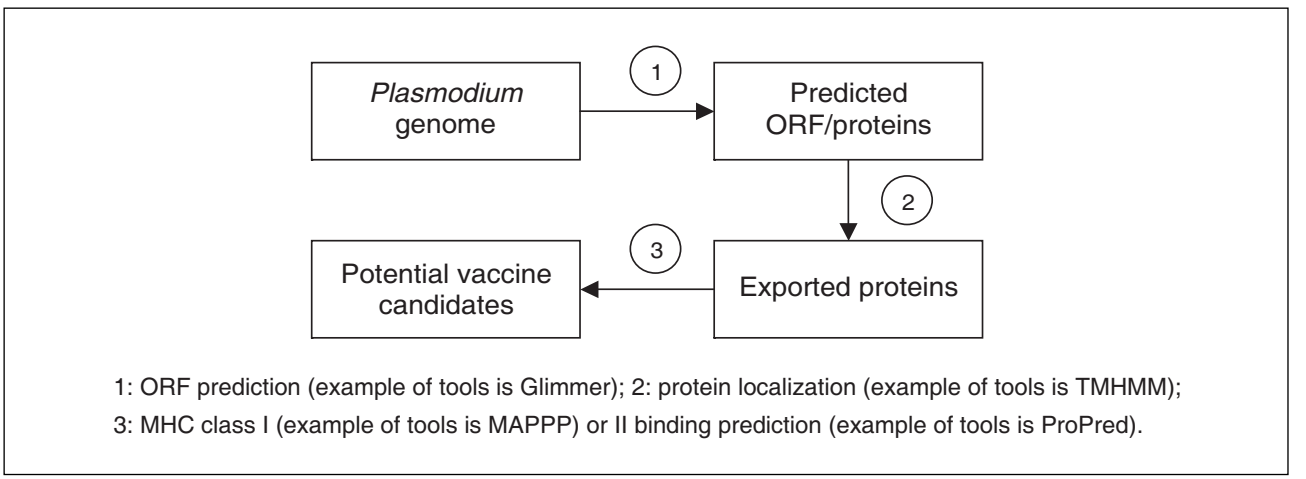

Fig. 1. Process for predicting proteins regarded as potential vaccine candidates from the *Plasmodium* genome sequence.

7. Capecchi, B., Serruto, D., Adu-Bobie, J., Rappuoli, R., Pizza, M. *The genome revolution in vaccine research.* Curr Issues Mol Biol 2004, 6: 17-27.

8. De Groot, A.S., Rappuoli, R. *Genome-derived vaccines.* Expert Rev Vaccines 2004, 3: 59-76.

9. De Groot, A.S., Sbai, H., Aubin, C.S., McMurry, J., Martin, W. *Immuno-informatics: Mining genomes for vaccine components.* Immunol Cell Biol 2002, 80: 255-69.

10. Grandi, G. *Rational antibacterial vaccine design through genomic technologies.* Int J Parasitol 2003, 33: 615-20.

11. De Groot, A.S. *Immunomics: Discovering new targets for vaccines and therapeutics.* Drug Discov Today 2006, 11: 203-9.

12. Serruto, D., Rappuoli, R. *Post-genomic vaccine development.* FEBS Lett 2006, 580: 2985-92.

13. Scarselli, M., Giuliani, M.M., Adu-Bobie, J., Pizza, M., Rappuoli, R. *The impact of genomics on vaccine design.* Trends Biotechnol 2005, 23: 84-91.

14. Mora, M., Veggi, D., Santini, L., Pizza, M., Rappuoli, R. *Reverse vaccinology.* Drug Discov Today 2003, 8: 459-64.

15. Masignani, V., Rappuoli, R., Pizza, M. *Reverse vaccinology: A genome-based approach for vaccine development.* Expert Opin Biol Ther 2002, 2: 895-905.

16. Dhiman, N., Bonilla, R., O'Kane, D.J., Poland, G.A. *Gene expression microarrays: A 21st century tool for directed vaccine design.* Vaccine 2001, 20: 22-30.

17. Grandi, G. *Rational antibacterial vaccine design through genomic technologies.* Int J Parasitol 2003, 35: 615-20.

18. Barry, M.A., Howell, D.P., Andersson, H.A., Chen, J.L., Singh, R.A. *Expression library immunization to discover and improve vaccine antigens.* Immunol Rev 2004, 199: 68-83.

19. Betton, J.M. *Rapid and highly efficient, the recombinational cloning technology is largely used for structural genomics and functional proteomics.* Biochimie 2004, 86: 601-5.

20. Dieckman, L.J., Hanly, W.C., Collart, E.R. *Strategies for high-throughput gene cloning and expression.* Genet Eng (NY) 2006, 27: 179-90.

21. Davies, D.H., Liang, X., Hernandez, J.E. et al. *Profiling the humoral immune response to infection by using proteome microarrays: High-throughput vaccine and diagnostic antigen discovery.* Proc Natl Acad Sci USA 2005, 102: 547-52.

22. Zagursky, R.J., Russell, D. *Bioinformatics: Use in bacterial vaccine discovery.* Biotechniques 2001, 31: 636, 638, 640.

23. Schonbach, C., Nagashima, T., Konagaya, A. *Textmining in support of knowledge discovery for vaccine development.* Methods 2004, 34: 488-95.

24. Flower, D.R., McSparron, H., Blythe, M.J. et al. *Computational vaccinology: Quantitative approaches.* Novartis Found Symp 2003, 254: 102-20.

25. Brusic, V., August, J.T., Petrovsky, N. *Information technologies for vaccine research.* Expert Rev Vaccines 2005, 4: 407-17.

26. Flower, D.R. *Databases and data mining for computational vaccinology.* Curr Opin Drug Discov Devel 2003, 6: 396-400.

27. Blythe, M.J., Doytchinova, I.A., Flower, D.R. *JenPep: A database of quantitative functional peptide data for immunology.* Bioinformatics 2002, 18: 434-9.

28. Guan, P., Doytchinova, I.A., Zygouri, C., Flower, D.R. *MHCPred: A server for quantitative prediction of peptide-MHC binding.* Nucleic Acids Res 2003, 31: 3621-4.

29. Reche, P.A., Zhang, H., Glutting, J.P., Reinherz, E.L. *EPIMHC: A curated database of MHC-binding peptides for customized computational vaccinology.* Bioinformatics 2005, 21: 2140-1.

30. Vivona, S., Bernante, F., Filippini, F. *NERVE: New Enhanced Reverse Vaccinology Environment.* BMC Biotechnol 2006, 6: 35.

31. Hertz, T., Yanover, C. *PepDist: A new framework for protein-peptide binding prediction based on learning peptide distance functions.* BMC Bioinformatics 2006, 7(Suppl. 1): S3.

32. *PlasmoDB: An integrative database of the Plasmodium falciparum genome. Tools for accessing and analyzing finished and unfinished sequence data. The Plasmodium Genome Database Collaborative.* Nucleic Acids Res 2001, 29: 66-9.

33. Fletcher, C. *The Plasmodium falciparum Genome Project.* Parasitol Today 1998, 14: 342–4.

34. Bahl, A., Brunk, B., Coppel, R.L. et al. *PlasmoDB: The Plasmodium genome resource. An integrated database providing tools for accessing, analyzing and mapping expression and sequence data (both finished and unfinished).* Nucleic Acids Res 2002, 30: 87-90.

35. Verma, P., Sharma, Y.D. *Malaria genome project and its impact on the disease.* J Vector Borne Dis 2003, 40: 9-15.

36. Hoffman, S.L., Rogers, W.O., Carucci, D.J., Venter, J.C. *From genomics to vaccines: Malaria as a model system.* Nat Med 1998, 4:1351-3.

37. Coppel, R.L. *Bioinformatics and the malaria genome: Facilitating access and exploitation of sequence information.* Mol Biochem Parasitol 2001, 118: 139-45.

38. Johnson, J.R., Florens, L., Carucci, D.J., Yates, J.R. 3rd. *Proteomics in malaria.* J Proteome Res 2004, 3: 296-306.

39. Cooper, R.A., Carucci, D.J. *Proteomic approaches to studying drug targets and resistance in Plasmodium.* Curr Drug Targets Infect Disord 2004, 4: 41-51.

40. Petrovsky, N. *Immunome research.* Immunome Res 2005, 1: 1.

41. Brusic, V. *From immunoinformatics to immunomics.* J Bioinform Comput Biol 2003, 1: 179-81.

42. De Groot, A.S. *Immunomics: Discovering new targets for vaccines and therapeutics.* Drug Discov Today 2006, 11: 203-9.

43. Gardner, M.J. *The genome of the malaria parasite.* Curr Opin Genet Dev 1999, 9: 704-8.

44. Doolan, D.L., Aguiar, J.C., Weiss, W.R. et al. *Utilization of genomic sequence information to develop malaria vaccines.* J Exp Biol 2003, 206: 3789-802.

45. Safitri, I., Jalloh, A., Tantular, I.S. et al. *Sequence diversity in the amino-terminal region of the malaria-vaccine candidate serine repeat antigen in natural Plasmodium falciparum populations.* Parasitol Int 2003, 52: 117-31.

46. Ferreira, M.U., Ribeiro, W.L., Tonon, A.P., Kawamoto, F., Rich, S.M. *Sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-1 (MSP-1) of Plasmodium falciparum.* Gene 2003, 304: 65-75.

47. Zakeri, S., Dinparast Djadid, N., Zeinali, S. *Sequence heterogeneity of the merozoite surface protein-1 gene (MSP-1) of Plasmodium vivax wild isolates in southeastern Iran.* Acta Trop 2003, 88: 91-7.

48. Di Girolamo, F., Raggi, C., Bultrini, E. et al. *Functional genomics, new tools in malaria research.* Ann Ist Super Sanita 2005, 41: 469-77.

49. Kappe, S.H., Gardner, M.J., Brown, S.M. et al. *Exploring the transcriptome of the malaria sporozoite stage.* Proc Natl Acad Sci USA 2001, 98: 9895-900.

50. Young, J.A., Fivelman, Q,L., Blair, P.L. et al. *The Plasmodium falciparum sexual development transcriptome: A microarray analysis using ontology-based pattern identification.* Mol Biochem Parasitol 2005, 143: 67-79.

51. Chiang, P.K., Bujnicki, J.M., Su, X., Lanar, D.E. *Malaria: Therapy, genes and vaccines.* Curr Mol Med 2006, 6: 309-26.

52. Haddad, D., Bilcikova, E., Witney, A.A. et al. *Novel antigen identification method for discovery of protective malaria antigens by rapid testing of DNA vaccines encoding exons from the parasite genome.* Infect Immun 2004, 72: 1594-602.

53. Aguiar, J.C., LaBaer, J., Blair, P.L. et al. *High-throughput generation of P. falciparum functional molecules by recombinational cloning.* Genome Res 2004, 14: 2076-82.