HUMAN VACCINES & IMMUNOTHERAPEUTICS
2020, VOL. 16, NO. 3, 723–735
https://doi.org/10.1080/21645515.2019.1670035

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REVIEW

Computational tools for modern vaccine development

Sunita[a,b], Andaleeb Sajid[c], Yogendra Singh[b], and Pratyoosh Shukla[a]

[a]Enzyme Technology and Protein Bioinformatics Laboratory, Department of Microbiology, Maharshi Dayanand University, Rohtak, India; [b]Bacterial Pathogenesis Laboratory, Department of Zoology, University of Delhi, Delhi; [c]National Institutes of Health, National Cancer Institute, Bethesda, MD, USA

ABSTRACT

Vaccines play an essential role in controlling the rates of fatality and morbidity. Vaccines not only arrest the beginning of different diseases but also assign a gateway for its elimination and reduce toxicity. This review gives an overview of the possible uses of computational tools for vaccine design. Moreover, we have described the initiatives of utilizing the diverse computational resources by exploring the immunological databases for developing epitope-based vaccines, peptide-based drugs, and other resources of immunotherapeutics. Finally, the applications of multi-graft and multivalent scaffolding, codon optimization and antibodyomics tools in identifying and designing *in silico* vaccine candidates are described.

ARTICLE HISTORY
Received 7 June 2019
Revised 28 August 2019
Accepted 13 September 2019

KEYWORDS
Vaccine; reverse vaccinology; rational design; antibodyomics tools; epitope prediction; multi-graft scaffolding

1. Introduction

Vaccine design is a complicated process, however, advances in bioinformatics will probably make vaccine design and drug development easy.[1] The design of vaccines can be divided into two broad categories: the traditional and the modern approach. The design of traditional vaccines is expensive, time-consuming, and not applicable for antigenically diverse pathogens.[2] This is because of the genetic/antigenic diversity of pathogens, insufficient information about the interaction between pathogen and host, absence of a permissive cell line, and lack of successful animal models.[3,4] It has been observed that vaccine development for severe diseases, such as smallpox, human immunodeficiency virus causing acquired immunodeficiency syndrome (HIV-AIDS), and tuberculosis (TB) was also affected by these drawbacks.[3] On the other hand, vaccines developed by the traditional approach for smallpox, polio, and diphtheria have several drawbacks and faced many problems.[5]

Due to limitations of the conventional technology, modern technologies have come into existence, including recombinant DNA technology, rational vaccinology, structural biology, conjugate vaccines, next-generation technology and epitope-based vaccine design. With the help of recombinant DNA technology, vaccines developed are regarded as safe, effective and inexpensive as compared to other traditional vaccines and apply for the bulk production of sub-unit vaccines.[6] Several *in silico* tools have been designed for the development of immunotherapy along with peptide-based drugs discovery in the previous two decades. Therefore, it is crucial to develop novel therapeutics with prophylactic vaccines and computational tools against different diseases like malaria, HIV-AIDS, and tuberculosis.[7] Practice is required in the field of genomics, structural biology, computational biology, and rational vaccinology to improve the development of vaccines.[8] Initiation of sequence analysis and recombinant DNA technology (RDT) opened the way to innovative vaccine design, including the concept of epitope-based vaccine design. The genomic analysis of pathogens also facilitates the classification and recognization of the protective epitope.[9]

Modern computational design starts as a dynamic force to facilitate structural vaccinology, whereby protein antigens are designed to prepare novel biomolecules with better immunological properties.[10] Regular progress in vaccine development and diagnostic fields accelerate the broad application of structural vaccinology (SV), reverse vaccinology (RV) and antigen recognition technology.[11] However, systems biology aids in predicting the host-pathogen interactions, and improves adjuvant capability to provide long-lasting immunity.[12] In these novel technologies, rational vaccinology is an innovative and functionally applicable approach to design the potent immunogen for the induction of prolonged protective immunity. With the help of this technology, synthetic peptide vaccine was designed for the treatment of asthma.[13] The comprehensive vaccines for viral pathogens such as HIV, influenza and hepatitis C virus may be designed through rational vaccinology approach as reported by Burton, 2017.[14] Antigen prediction is an important criterion in the process of vaccine development. Vaxi Jen is an online software, based on the alignment-free approach and can directly predict the antigens.[15] It is the first online server for alignment-independent prediction of protective antigens. The modern technology of vaccine design also includes reverse vaccinology, which accelerates the process of vaccine development.[16] Epitope mapping is also a crucial factor in designing an effective vaccine as it generates vigorous reactions from both B cells and T cells and *in silico* prediction successfully increases the epitope prediction.[17,18] A multi-epitope peptide vaccine was developed to stimulate an effective immune response for the treatment of brucellosis. Ren et al., 2019[19] prepared a multi-epitope vaccine through bioinformatic

CONTACT Pratyoosh Shukla pratyoosh.shukla@gmail.com Enzyme Technology and Protein Bioinformatics Laboratory, Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana 124001, India

tools for evaluating its immune response in mice, and high production of IgG antibodies was observed.

Broadly neutralizing antibodies (bNAbs) is a new term in immuno-informatics and is still in the computational pipeline. It was initially applied to analyze a different class of HIV-1 bNAbs entirely based on 454-sequencing method.[20] These antibodies have the feature of targetting only conserved epitopes of the microbes that play a significant role in virulence[21] and develops a new area of research to design a vaccine against quickly mutating viruses such as HIV and influenza.[22] The proper implementation of computational tools minimizes the various challenges in the field of vaccine development. Computational biology also constitutes side-chain prediction tools to design an antibody and predict its structure.[23] Different aspects of multigraft, multivalent scaffolding, codon optimization, and antibodyomics tools to identify and design potential vaccine candidate are also well described. This review provides relevant information about the latest computational tools that are essential for vaccine design since all of them have a unique feature and applcation according to the need of the situation.

2. Vaccine design: systems biology and structural antigen design

Vaccines not only arrest the beginning of different diseases, but also assign a doorway for its elimination and help in reducing the toxicity.[24] Systems biology and structure-based antigen design are novel techniques to develop vaccines. A biological system is thoroughly analyzed via systematically including diverse areas such as genetics, biology, and chemistry. It gives valuable information about the gene, protein, and different metabolic pathway involved in pathogenesis.[25] Systems biology collects a massive amount of biological data from the various hierarchical levels. The information about protein expression levels, DNA sequences, RNA, microRNAs, metabolite biology, protein-protein and protein-DNA interactions are obtained from the various biological datasets.[26] The data generated will be further integrated and formulated through mathematical models to explain the structure of the system. Additionally, it helps in the analysis of the sequences of genes and proteins involved in the virulence in different microbes. The progress of "omics" technologies such as proteomics, genomics, metabolomics, and transcriptomics offers a comprehensive study of systems biology.[27] Proteomics play an essential role in the field of vaccine design including immunogenic techniques along with a genome-based approach and to discover the potent immunogenic protein.[28] Proteomic experiments in microorganisms were verified by whole genome sequencing and bioinformatics tools to discover new vaccines.[29] Therefore, computational biology is an essential factor to fulfill this approach.

The detection of efficient biomarkers is the primary aim of molecular medicine. Systems biology has significantly identified the biological markers used for the diagnosis of various diseases.[30] Groves et al., 2018[31] reported that systems biology enhances the recognition of a radiation-specific biomarker. Muhammad et al., 2019[32] showed that a computational approach, including systematic simulation-based meta-analytical framework, successfully predicted biomarkers. Oh et al., 2018[33] also demonstrated that the systems biology approach could play an essential role in designing potent vaccines against different diseases like Ebola or Zika virus (ZIKV), dengue, avian influenza and thrombocytopenia syndrome (SFTS).

Computational design is emerging as a driving force for structural vaccinology, where protein antigens are designed to produce new biomolecules to enhance immunological properties.[34] Structural vaccinology induces a higher protective immune response, thus aiding in discovering novel antigens.[35] The production of the vaccine can be enhanced by stabilizing the structure of complex antigens. Kaufmann and Flechtner[36] mentioned that Herpes Simplex Virus (HSV) vaccine could be rationally developed as an alternative option for the treatment of the disease. Bajic et al., 2019[37] reported that V_H1-69-encoded antibodies against influenza virus, HIV-1, and HCV. The mentioned antibodies have similar genetic and structural characteristics, and neutralize a wide spectrum of viral strains. Trobaugh et al., 2019[38] suggested that encephalitis virus vaccine could be designed by the implantation of structural vaccinology approaches, which is also referred to as rational vaccinology.

3. Adjuvants in vaccine design

An adjuvant can be defined as a vaccine component that enhances host immune response and plays an essential role in the development of vaccine.[39] Adjuvants made of aluminum were used 90 years ago to enhance the immune response of the vaccines. Different aluminum salt products are used in vaccines that have a special feature of immunopotentiation along with safety records.[40] Basically, aluminum hydroxide and aluminum phosphate are the two types of aluminum adjuvants used in specific licensed vaccines.[41] They are prepared by vaccine companies and can be easily purchased by manufacturers like Brenntag Chemtrade, Biosector, and SPI Pharma. Generally, they can be simply recognized through their trade names like Alhydrogel, Rehydragel, and Adju-Phos. Imject™ Alum made of amorphous aluminum hydroxycarbonate and crystalline magnesium hydroxide were used for preclinical and experimental studies.[42] Calabro et al., 2013[43] reported that MF59 is a potent adjuvant that recruits CD11b+ blood mononuclear cells in the mouse muscle. MF59 is used in clinical trials as a component in prophylactic and therapeutic vaccines of infectious disease, cancer, and allergies. It also stimulates different immune cells such as neutrophils, eosinophils, macrophages, and monocytes. Adjuvants can be designed by incorporating various components such as TLR4 agonist, flagellin, and T-helper agonists.[44] Kanzler et al, 2007[45] demonstrated that Toll-like receptors (TLRs) can enhance immune response, and thus can be used as vaccine adjuvant. TLR ligand-based adjuvants produce a robust immune response in the signaling of MyD88 in macrophages.[46]

Aucouturier et al., 2002[47] reported that montanide ISA 720 and 51 are used as water-in-oil emulsion adjuvants for human vaccine development. The TLR4 agonist, glucopyranosyl lipid adjuvant (GLA), protected mouse-adapted Ebola virus (ma-EBOV) and was prepared in a stable emulsion (SE) to stimulate immunogen and promote durable protection. Different adjuvants such as virosome, MPL and MF59 are applied in the design of vaccines like Invivac, Fendrix, and Pandemic Influenza vaccines, respectively.[48] MPL is the foremost and

only TLR ligand in licensed human vaccines, in the form of AS04 used for allergy treatment. This adjuvant is derivative of a liposaccharide that shows a reduced toxicity and maintains major immunostimulatory reaction of lipopolysaccharide.[49] No harmful effects of MPL were observed in the rabbits when weekly doses were administered. In addition, it does not show any adverse effect on respiratory function, reproduction or genotoxicity. QS-21 induced antigen-specific antibody responses, including $CD8^+$ T-cell response in mice and maintained a balanced production of IgG1 and IgG2a as compared to aluminum hydroxide that significantly favors IgG1 production.[50] It can be used as an efficient adjuvant against feline leukemia virus (FeLV) in the form of a recombinant retroviral sub-unit vaccine. Many databases are available to find the adjuvant, such as Vaxjo (http://www.violinet.org/vaxj).[51] This database incorporates approximately 400 vaccines that use an adjuvant and contains more than 100 vaccine adjuvants. Additionally, vaccine adjuvant design includes database development, omics bioinformatics, data analysis, and literature mining.[52]

4. Rational vaccine design

Rational vaccine design is an innovative approach in the field of vaccinology and is applied to design potent immunogens for the induction of prolonged protective immunity.[53] Generally, rational vaccinology is applicable for viral pathogens such as hepatitis C virus, HIV, and influenza.[54] The rationally developed vaccine consists of antigens, its delivery systems and an adjuvant to stimulate an immune response against specific epitopes of a particular pathogen. Computational modeling is an efficient tool to design the structure of a protein that can be determined through template-based and free modeling. Template-based modeling is entirely based on the 3D structure of a protein consisting of a selection of templates, sequence alignment, models construction, quality estimation, and structural modification.[55] Vaccine design based on a protein structure depends on the conserved sites present on pathogens and the neutralizing antibodies with conserved sites.[56] These neutralizing antibodies are adequate to inactivate antibodies and also induces prolonged protective immunity.

Vaxi Jen, the online software for the prediction of the antigens, can perform alignment-independent prediction of protective antigens and allows antigen classification separately based on the physicochemical properties of proteins. The server can be used on its own or in combination with alignment-based.[57] TLRs identify the pathogen and are responsible for inducing innate immunity. Peta-flops-scale supercomputers can be used for modulation, screening, and identification of new lead structures for hTLR4. Moreover, they are applied for cancer immunotherapy to design polymeric hybrid micelles.[58] With the incorporation of this technology, 12 compounds associated with tryptamine were screened and developed by *in silico* tools to preserve their molecular geometry while interacting with the hTLR4 binding site.[59] RDT is successfully applied in rational vaccinology for the production of chimeric proteins. The classification of these proteins was performed by the help of Expasy ProtParam. A synthetic peptide vaccine was developed to enhance the efficiency of antigen presentation to stimulate the humoral immune response and thus help in the treatment of asthma as reported by Hayman et al., 2018.[60]

Rational vaccinology has been applied to discover a novel synthetic peptide vaccine for the treatment of asthma. Researchers described that a peptide of interleukin-13 was expressed and upregulated in asthma, and this could serve as a crucial antigen to use for screening of asthma through experimental and computational methods.[61]

Human astrovirus can cause viral diarrhea, especially in children and immune-compromised patients, and still, there is no vaccine available to prevent this infection.[62] The rational vaccine design technology could be carried out for the development of a vaccine against it. Martinez et al., 2017[63] reported that cancer vaccines derived from MUC1-glycopeptides by implementing rational design technology. Different approaches are mentioned in Figure 1 for the rational vaccine design. The VBRC NERVE is a novel tool used to determine how specific proteins can act as potential vaccine candidates.[64] Furthermore, the Promoter Scan can recognize potential epitopes that are suitable for immune response and the expression of the gene.[9]

Bioinformatics approaches, such as structural approaches, MD simulations, and docking are also applied for the development of vaccines. It was reported that Chimeric Simian-Human Immunodeficiency Viruses (SHIVs) act as anti-HIV Env interventions in nonhuman primate (NHP) models and are designed by rational technology.[65] Infection by *Staphylococcus aureus* causes high mortality and morbidity in humans. Additionally, Kailasan et al., 2019[66] reported that Leukocidin AB could be rationally designed as a toxoid vaccine against this infection. Tai et al., 2019[67] also reported that Zika virus sub-unit vaccine can be rationally designed with high efficiency in which envelope protein domain III (EDIII) is engineered to be used as a vaccine candidate. Trobaugh et al., 2019[38] showed that a rational approach is applied for the attenuation of eastern equine encephalitis virus (EEEV), a mosquito-transmitted alpha virus.

5. Computational tools for vaccines development

The biological information generated in genetics, biotechnology, and molecular biology is well organized and stored with the help of bioinformatic tools.[68] Use of computational tools prior to lab experimentation is more advantageous as they are cost effective and take less time to operate. Immuno-informatics is a novel term applied to the conversion of large-scale immunological data in a compact form through the combination of computational and mathematical approaches.[69] These tools are based entirely on statistical and machine learning systems and are well established in analyzing and modeling molecular interactions during antigen presentation and processing. In Figure 2, different computational tools, for the development of vaccines, along with their software, are mentioned.

5.1. Side-chain and backbone modeling tools

Different types of receptors known as side chains are present on the cells and perform their function as gatekeeper of the

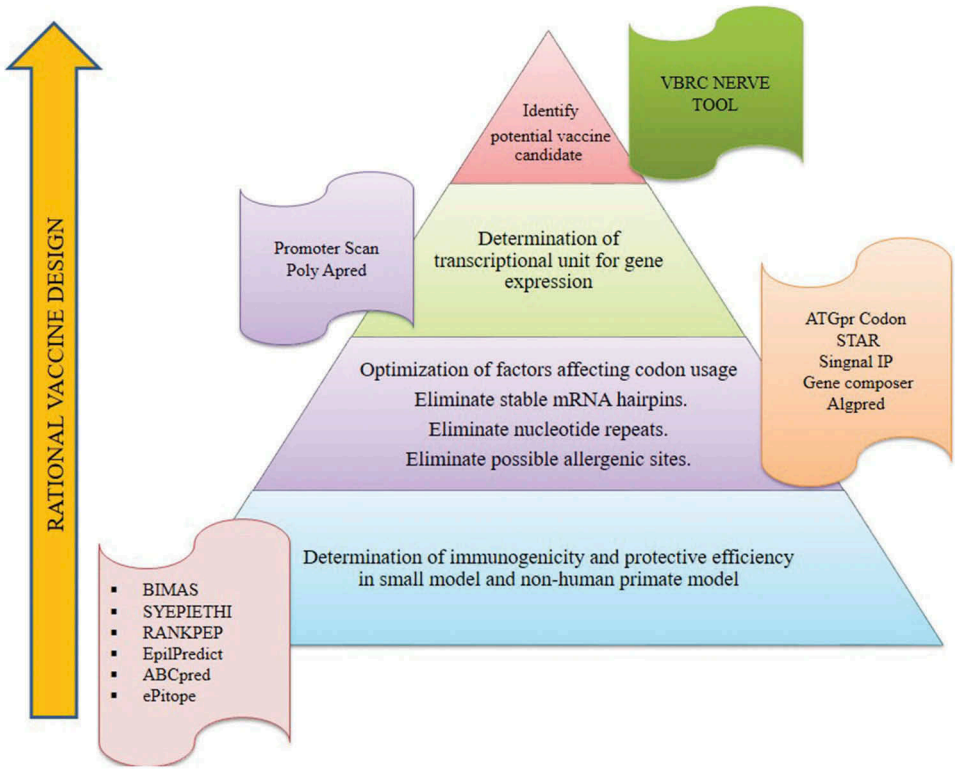

Figure 1. Rational vaccinology and its procedure to design vaccines through the VBRC NERVE tool. Illustration showing the steps involved in the identification of potential vaccine candidates through rational vaccinology. Each step uses different bioinformatics tools which are mentioned.

cells. Each side chain has its own characteristic structure, and only the substance identical to them can enter the cell. The side-chain prediction is an essential constituent of computational biology for designing and predicting antibody structure.[70] SCWRL and SCAP are tools which have been applied efficiently for modeling; they are used to determine and analyze mutations in protein side chains *in silico*. Moreover, these modeling tools can be applied for recognition and optimization of specific antibodies along with its affinity toward a particular target.[71] Additionally, a powerful modeling program is available to design glycan epitopes on immunogens.[72] Antibody modeling tools can be operated by backbone-dependent rotamer libraries.

Significantly, backbone modeling tools help in the modification of different antibodies. Leem et al., 2018[73] described position-dependent antibody rotamer swapper (PEARS, http://opig.stats.ox.ac.uk/webapps/pears), a side chain predictor which uses the IMGT position-dependent distribution of rotamers. It performs the side-chain prediction in less than 10 seconds.[74] Additionally, DRAGON and GADGET are the antibodies use protein folding programs used to predict the secondary structure and ligand-binding site of the proteins. RAMBLE is an additional bioinformatics analysis tool with different permutations to check the connectivity of disulfide bonds, chain topology, and tryptone side-chain alignment.[75] RAPPER is an *in silico* approach to generate 3D modeling of proteins for comparative analysis with a high degree of accuracy. It can be used to identify target sequences by exploring the conformational structure of a protein.[76]

5.2. Multi-graft and multivalent scaffolding

Multi-graft and multivalent scaffolding are the prominent approaches for the development of vaccines. A multivalent ligand has multiple copies of ligands which are capable of binding to different sites of the receptor.[77] The concept of scaffolding expands the view of vaccine design based on epitope engineering. The implementation of epitopes of interest executed to the scaffolds of heterologous proteins was proven by studying HIV-1, flu, and RSV by Walenskey and Bird, 2010.[78] Multivalent scaffolding technology is applied to epitope vaccine design based on the fact that a scaffold is present in the protein of interest. Perhaps it can be considered that multivalent scaffolds presents an antigen in a highly ordered and repetitive manner to induce a strong immune response. Furthermore, Ullah et al., 2019[79] reported that an inhibitor of the scaffold protein RACK1 (Receptor for Activated C Kinase 1) could inhibit the proliferation of HSV. The virus-like particles (VLPs) are small biological structures consisting of viral proteins similar to virion but not having genetic material and is incapable of stimulating an immune response. Barwal et al., 2016[80] also defined the virus-like particles as an attractive nano-particulate scaffold to apply in biological science and medicine. Hill et al., 2018[81] also mentioned that VLPs could be engineered to develop as antigens and thus help in the drug discovery and delivery. Rynda et al., 2014[82] demonstrated that virus-like particles could be used to make a vaccine candidate by exploring the lung as a site for immunostimulation. Prediction of protein structure is performed through composite modeling, which uses multiple templates for the development of multi-graft scaffold immunogen.

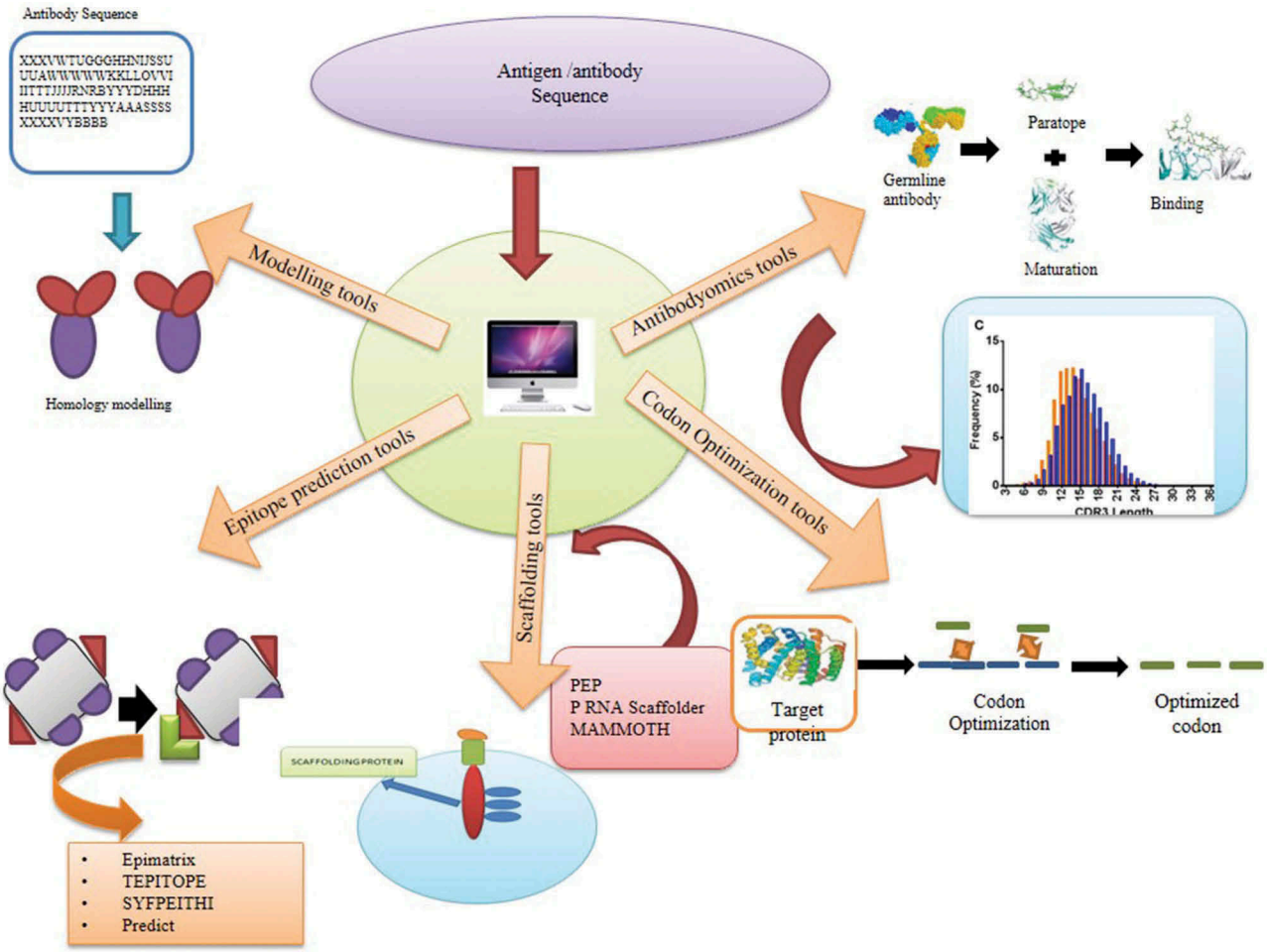

Figure 2. Computational strategies for the development of potent vaccine including antigen processing. Illustration showing the processing and screening of the antigen as an immunogen. The pathogen enters the cell and secretes proteins, which can be predicted by the Mature P software. Further processing of the protein is performed by proteosome and the antigenic peptide is released. This peptide, capable of binding MHC, can be predicted by MHCPred, RANKPEP, and SVMHC software. The peptide is then targeted as an epitope by the epitope prediction tools (IEDB, EpiJen, ProPred) and is selected as a potential vaccine candidate.

Identification of a specific scaffold having multiple epitopes is challenging, antibodies choose these epitopes without any hinderance.[83] Küry et al., 2017[84] demonstrated that a proteosome scaffold subunit functions only during the phase of development of neurogenesis, and also showed that PSMD12 variants might affect neurodevelopment. Hence it can be said that protein engineering helped in the development of novel diagnostics and therapeutic agents. Different protein scaffolds such as DARPins (designed ankyrin repeat proteins) cysteine knots can be used as a scaffold to represent a functional site.[85] Multivalent interaction of biological molecules is performed in different biochemical events to enhance the binding affinity, the avidity, and specificity of the ligand to the receptor. Hence multivalent ligands could be an alternate way to treat diseases as reported by Greenspan and Cavacini, 2019.[86]

A multigraft interface is a novel approach applied to graft epitopes so that antibody binding specificity can be improved and thus potentially affect the nature of antibodies.[87] It is also applicable for the engineering of novel epitope scaffolds that exhibit neutralizing antibody 2F5 of HIV-1and also deal with the CDR H3 antibody loop. Gourlay et al., 2017[88] presented an automated computational tool, SAGE (strategy in alignment and grafting of epitopes), for the insertion of immune-generating epitopes onto a given scaffold. The approach assigns the identification of a graft position on any target antigen with a known three-dimensional structure, which is fast, extensive, and efficient tool. Mishra et al., 2018[89] reported prime scanning of epitope grafting and studied about computational grafting of malarial epitopes in serum albumin.

5.3. Antibodyomics tools

Antibodyomics is an essential computational tool initially applied for the analysis of the different classes of HIV-1 bNAbs, and based entirely on 454-sequencing.[90] Significantly it is an innovative approach for the development of a vaccine against antigenically variable viruses. Antibodyomics tools consist of different phases such as putative germline genes, error correction, and comparison of different bNAbs.[91] The variable chains of the antibody have complementary-determining regions (CDR) where these bNAbs specifically bind. Additional information can be obtained to create a standard database through CDR3 analysis. This analysis helps to determine germline precursors and intermediate immunoglobulins from an NGS-derived repertoire. There are two analytical parameters on which it is based; the first

is the identification of a sequence to a known bNAb (Y-axis), while the second one includes the analysis of the divergent sequences of putative germline genes (X-axis). The graph plotted between the two axes, and it was observed that closely linked somatic variants form 'clusters' which are different from the main sequence population.[92] Furthermore, these variants are recognized with the intra-donor phylogenetic analysis and help in searching the sequence that has a similar evolutionary pattern as template bNAbs. It is utilized for the *de novo* recognition of VRC01-like broad neutralizing antibodies from HIV-1-infected donors.

For the development of epitope vaccines, computational tools like structure-based immunogen design and a wide range analysis of antibody can be used.[93] Kwong et al., 2017[90] reported that neutralizing antibodies can be developed by understanding the information about genetics and immunological processes. Serum neutralization can also be performed to identify and quantify neutralizing antibodies.

5.4. Reverse vaccinology

Reverse vaccinology is a broad term applied to recognize potential vaccine candidates through analyzing the proteome of the pathogen with the help of computational tools. It is an essential technology for the mapping of epitopes and prediction of monovalent peptide vaccines to be used in the therapeutic processes.[94] Reverse vaccinology is advantageous as it analyzes the complete genome of the pathogen and specifically chooses proteins that act as a potential antigen. Vaxign is a computational approach used to predict ideal vaccine candidates and develop distinct vaccines against proteins responsible for antibiotic resistance in the pathogens.[95] Additionally, this technology is appropriate for the screening of the antigenic peptide in different pathogens like *Neisseria meningitides*, Group B Streptococcus (GBS), and *Porphyromonas gingivalis*.[96] Functionally, NERVE program is an essential tool used for the development of reverse vaccinology. In this technology, open-reading frames (ORFs) play a significant role and screening of potential ORFs can be performed by the NERVE program.[97] The computational analysis helps in the identification and localization of sub-cellular proteins having adhesion-like properties. HensBC can determine and assemble ORFs of different proteins (mitochondrial, cytoplasmic, nuclear, or extracellular) with 80% efficiency to make them a potential vaccine candidate.[9]

The identification of the pathogenic genome and complete screening of open reading frames (ORFs) is performed to select the specific sequence of the peptides responsible for an immunogenic response. Incorporation of *in silico* tools such as GLIMMER, ORF-FINDER, and GS-Finder will help scan the whole genome of the pathogenic strain. Following scanning, the identification of therapeutic proteins is carried out by various *in silico* tools such as ProDom, Pfam, and PROSITE. Initially, reverse vaccinology was applied for the development of Group B *meningococcus* vaccine by applying different software programs. Yee, 2019[98] reported that EV-A71 is a next-generation vaccine candidate against Enterovirus A71 which is responsible for causing foot and mouth diseases and severe neurological complications. Implementation of reverse genetics technology can be used to develop an rgEV-A71 strain to act as a potential vaccine candidate. It is difficult to control *Ctenocephalides felis* and disease risks associated with them, hence Contreras et al., 2018[99] suggested that the reverse vaccinology approach could be applied for the development of a novel vaccine against it. Moreover, this technique also helps in the identification of MHC Class-II-restricted epitopes from *Leishmania donovani* against visceral leishmaniasis.[100] It can be successfully applied for the designing of novel vaccines against serogroup B *Neisseria meningitidis*, which is a cause of meningitis.[101]

Nosocomial infection is mainly caused by *Pseudomonas aeruginosa* in immuno-compromised patients, and recently three proteins, PSE17-1, PSE41-5, and PSE54, were identified by the application of reverse vaccinology as potential vaccine antigens. These are novel lectins of *P. aeruginosa* and contribute a significant role in infecting host cells.[102] Naz et al., 2019[103] made the computational pipeline termed "PanRV" that applied for both pangenome and reverse vaccinology approaches. It includes four functional modules- Pangenome Estimation Module (PGM), Reverse Vaccinology Module (RVM), Functional Annotation Module (FAM), and Antibiotic Resistance Association Module (ARM). Multi-epitope subunit vaccine can be designed by incorporating reverse vaccinology against avian influenza A (H7N9) as reported by Hasan et al., 2019.[104] Reverse vaccinology has been successfully applied to categorize novel potential vaccine candidates against *Acinetobacter baumannii*, which is an evolving pathogen mainly found in intensive care units (ICU). Araujo et al., 2019[18] also discussed the success of omics and reversed vaccinology for the prediction of novel vaccine target in the whole genome of *Corynebacterium pseudotuberculosis*, which is an etiological agent of veterinary related diseases.

5.5. Codon optimization and other available software

The expression of proteins can be enhanced with a technology known as codon optimization, an approach in the field of immunoinformatics.[105] Different codon optimization algorithms are available for the high production of protein. Codon Optimization OnLine (COOL, http://bioinfo.bti.a-star.edu.sg/COOL/),[106] is a new tool that functions to synthesize genes. With the implementation of COOL, different codon optimization parameters including codon pairing, codon adaptation index, and specific codon usage, can be customized. An online application known as OPTIMIZER (http://genomes.urv.es/OPTIMIZER[107]) is built to optimize codon usage of a gene to increase its expression level.[108] However, OPTIMIZER can optimize strongly expressed genes in more than 150 prokaryotic species during the process of translational, thus, it can predict highly expressed genes.

CpG is another technique applied for the optimization of a codon and concerns CG dinucleotides. Studies have shown that the immune response of DNA vaccines could be raised by CpG motif engineering.[109] Narum et al., 2001[110] reported the protein expression of DNA vaccines could be enhanced by the optimization of gene fragments that code for *Plasmodium falciparum* merozoite proteins, which ultimately raised immunity in mice. Stachyra et al., 2016[111] also showed that codon optimization can be used to make potential DNA vaccines for

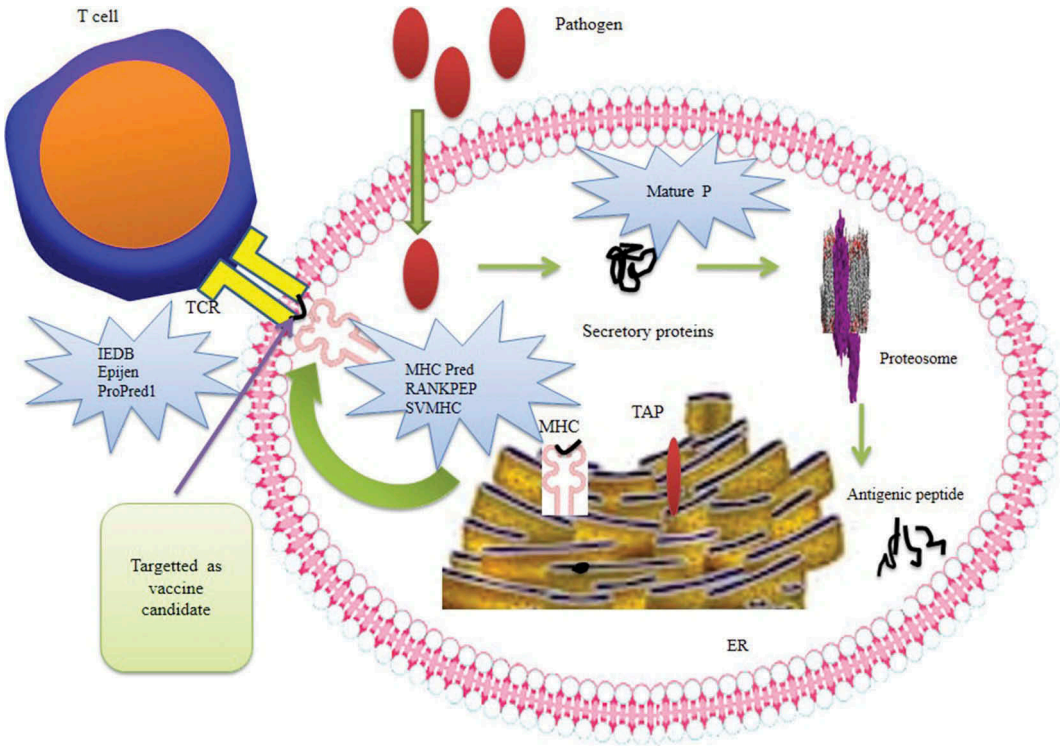

Figure 3. A review of applied computational methods for designing vaccines. Illustration showing various computational tools that help in the development of a vaccine. The antibodyomics tools modify the antibody to enhance binding to the antigen. Codon optimization tools such as Codon Optimization Online (COOL), OPTIMIZER, and Codon Wizard optimize the codon for enhanced gene expression. Epimatrix and TEPITOPE SYFPEITHI are scaffolding and epitope prediction tools. Modeling tools such as CHARMm, MacroModel, and MOIL are used for modeling the structure of the protein.

avian influenza virus H5N1 in chickens and mice. Pattern recognition receptors (PRRs) are the components of the innate immune system used to differentiate DNA of prokaryotes from eukaryotes. They use 'CpG dinucleotide motifs' in base specific context and do not disturb codon optimization.

Further modification of the sequence of a protein, DNA and RNA can be performed to predict the codon usage via a combinatory algorithm. Remarkably the optimized codon is used in *E. coli* for the expression of recombinant TEV-protease.[112] Gao et al., 2004[113] explained the advanced application of GUI software applied to optimize and break the open reading frame (ORF) of giving DNA into triplets. CodonWizard is an also automatic software program for modification of codon optimization and freely available for scientists.[114]

5.6. Epitope prediction tools

The epitope is the determinant of the antibody attachment site on the part of antigen and is recognized through the host immune cells. An effective vaccine could be designed by the epitope prediction tools as the epitope stimulates immune reactions from both B cells and T cells.[115] Different computational tools such as support vector machines (SVMs), motif-based systems, QSAR (quantitative structure-activity relationship analysis), structure-based, neural networks, and Hidden Markov models (HMMs) approaches are used to analyze the peptide interactions.[116] Mapping of B cell and T cell epitopes is known as "epitope fishing", which can screen the potential epitope in a pathogen. Epitope mapping in the genome of *Mycobacterium tuberculosis* was reported and analyzed with the help of predictive algorithms.[117] Thousands of alleles are present on the A and B loci of HLAs. This group of alleles is termed as super type; they occasionally bind to the same set of peptides and contribute to vaccine design.[118] Different potential supertypes could be discovered through the scoring matrix of the position specified by the alignment of MHC-I peptides. Support Vector Machines (SVMs) are applied to discriminate data into two distinct groups, based on the statistical theory: the binders and non-binders.[119] However, the Hidden Markov model (HMM) helps find the sequences that have 'binder-like' qualities and also identify complicated peptide patterns through the implementation of a Bayesian neural network.

B-cell epitope prediction is performed by different methods such as hydrophilicity profile, flexibility profile, surface probability and HMM.[120] Antigen processing and its selection are also important criteria in vaccine design. The development of a potential vaccine candidate with the help of bioinformatics tools are explained in Figure 3. These bioinformatics tools could be successfully implemented for the prediction of protein epitope domains targeted via human $CD4^+$ T-cells.

There are different tools available for B-cell epitope prediction.[121] ElliPro is used to determine the presence of discontinuous and conformational epitopes. DiscoTope (http://www.cbs.dtu.dk/services/DiscoTope/)[122] predicts discontinuous B-cell epitopes by analyzing 3D protein structures and has been used to predict the epitope in Alkhumra hemorrhagic fever virus (AHFV). DiscoTope can also be used for designing drugs and peptide-based vaccine, and in the development of diagnostic

Table 1. Different types of vaccines and their developing strategies.

S.No.	Vaccine type	Pathogen	Disease	Strategy	Reference
1.	Multi-epitope based	Kaposi's sarcoma-associated herpesvirus	Kaposi sarcoma	Immuno-informatics	Chauhan et al., 2019[126]
2.	Multi-epitope vaccine	*Pseudomonas aeruginosa*	Nosocomial infections	Comparative proteomics	Solanki et al., 2019[127]
3.	DNA vaccine	Ebola virus	Ebola virus disease	Computer design; gene expression; immunogenicity	Bazhan et al., 2019[128]
4.	Multi-epitope Peptide Vaccine	*Neisseria gonorrhoeae*	Gonorrhea	*In-silico* hierarchical approach	Jain et al., 2016[129]
5.	Subunit vaccine	Marburg virus	Hemorrhagic fever (MHF)	Reverse vaccinology	Hasan et al., 2019[130]
6.	Epitope-based	Nairovirus	Crimean-Congo hemorrhagic fever (CCHF)	Molecular docking and dynamics methods	Nosrati et al., 2019[131]
7.	Peptide-based vaccine	*Providencia stuartii*	Purple urine bag syndrome	Reverse Vaccinology (RV)	Asad et al., 2018[132]
8.	Multi-epitope vaccine	Human papilloma virus (HPV)	Warts and cancer	Structural vaccinology	Negahdaripour et al., 2018[133]
9.	Next generation vaccines	*Echinococcus granulosus*	Cystic echinococcosis	Systems vaccinology and mathematical/computational modeling	Pourseif et al., 2017[134]
10.	Peptide vaccine	Human papillomavirus (HPV)	Cervical cancer	Immunoinformatics and structural vaccinology approaches	Doorbar et al., 2015[135]
11.	Multi-epitope peptide vaccine	*Brucella spp.*	Brucellosis	Immuno-informatics	Saadi et al., 2017[136]
12.	Epitope-Based Peptide Vaccine	Mokola Rabies Virus	Meningo-encephalo-myelitis	*In silico* Approaches	Mohammed et al., 2017[137]
13.	T cells with Chimeric Antigen Receptor (CAR)	Acute lymphoblastic leukemia	Acute lymphoblastic leukemia	Genetic engineering	Dokmanović et al., 2017[138]
14.	Peptide vaccine	HPV	HPV-associated cancer	Conventional with Immuno-informatics	Atherton et al., 2018[139]
15.	Dendritic Cell Vaccines	Cancer antigens	Cancer	Immuno-informatics	Doytchinova et al., 2018[140]
16.	DNA Vaccines	Papillomavirus (HPV)	Human Cervical cancer and cervical intraepithelial neoplasia (CIN)	Genetic immuno-therapy, pharmaco-logical tool	Cordeiro et al., 2018[141]
17.	Epitopes based	Zika virus	Guillain–Barré syndrome	In silico-predicted immunogenic	Makhluf et al., 2018[142]
18.	Epitopes based	*Vibrio anguillarum*	Vibriosis	Reverse vaccinology	Baliga et al., 2018[143]
19.	Multi-epitope subunit vaccine	Chikungunya virus	Chikungunya	Immuno-informatics	Narula, et al., 2018[144]
20.	Sub-unit vaccine	*Plasmodium falciparum* and *P. vivax*	Malaria	Rational design	Draper et al., 2018[145]
21.	Epitope based vaccine	Dengue virus	Dengue	Immuno-informatics and Molecular Docking	Shen et al., 2018[146]
22.	Multi-Epitope based vaccine	*Staphylococcus aureus*	Skin infections and food poisoning	Immuno-informatics and in silico approach	Hajighahramani et al., 2017.[147]

kits. The 3D-Epitope-Explorer (3DEX) software allows mapping of conformational epitopes using 3D structures of proteins based on an algorithm. Other prediction tools include CEP (conformational epitope prediction, http://bioinfo.ernet.in/cep.html), Hopp-Woods hydrophilicity, Kyte-Doolittle hydrophilicity, Eisenberg moment, Karplus-Schultz flexibilty, Emini surface probability, and the PROTEAN module of the LASERGENE software (DNASTAR, Inc, USA).[121] Poorinmohmmad et al., 2014[123] reported that Discotope could be successfully applying for the prediction of the conformational epitope in Alkhumra hemorrhagic fever virus (AHFV). Moreover, it can be utilized for designing of the drug, peptide-based vaccine, and development of the diagnostic kit. The 3D-Epitope-Explorer (3DEX) software allow mapping of conformational epitopes using 3D structures protein based on algorithm.[124]

The Artificial Neural Network (ANN) and Quantitative Matrices (QM) are the basis of nHLAPred, which is used for the prediction of MHC-I binding peptides. Whether 9-mer peptides would bind an MHC-I molecule or not will be predicted by the Kernel-based Inter-allele peptide binding prediction SyStem (KISS) in SVM. Different databases such as MHCBN, LANL, SYFPEITHI Parker hydrophilicity, BepiPred and Immune Epitope Database (IEDB; www.immuneepitope.org) are additional online tools for the prediction of B-cell epitopes[125].

Different types of vaccines, along with their development strategies based on epitope prediction under pipeline, are discussed in Table 1. There has been no successful vaccine against *Plasmodium vivax* until now. With the help of epitope prediction, the potential epitope on AMA-1 was identified and developed as a highly effective vaccine candidate.[148] Ren et al., 2019[19] developed a multi-epitope vaccine using bioinformatic tools and evaluated its immune response in mice. They observed a high production of IgG antibodies that protect against lethal doses of *Acinetobacter baumannii*. Trypanosomiasis is a tropical disease that is caused by the genus *Trypanosoma* and affects domestic animals and humans. Guedes et al., 2019[149] used *in silico* tools to predict and characterize B-cell epitopes for South American and African *T. vivax* strain to be used in diagnostics.

T-cell epitopes are essential for designing the vaccines as they play a vital role in the cellular response. These epitopes can be identified through T cell receptors from various cells including B-cells, CTLs etc.[150] Examples of T-cell epitope prediction tools are- BIMAS, IEDB, NetMHC, ProPred, TEPITPE, and CTLpred. Epijen is a freely available online software used for the prediction of T-cell epitopes and predicts epitopes based on quantitative matrices.[151] This approach can be applied to develop vaccines against HIV and malaria. T-cell epitopes can be designed by the use of recombinant DNA technology and bioinformatics tools alongside the knowledge of the genetic background of the pathogen and host immune response.[152] Recombinant DNA technologies make epitope-based vaccines more efficient, safe, and less expensive. Moutaftsi et al., 2006[153] reported that various CD8 + T-cell epitopes can be predicted in a vaccinia virus WR strain. Glanville et al., 2017[154] showed that the GLIPH algorithm accelerates the identification of T-cell epitopes by specifying T-cell receptor groups. Gutiérrez et al., 2016[155] validated the prediction of T-cell epitopes in the swine influenza model. The function of PigMatrix and its ability to differentiate between immunogenic and non-immunogenic peptides were also validated.

6. Conclusion and future perspective

In summary, vaccine development can be considered as one of the significant factors for global public health. The traditional techniques have several drawbacks for vaccine design, but the implementation of computational tools will overcome these limitations. Immunoinformatics approaches are more beneficial, and thus the demand for modern technologies such as reverse vaccinology, epitope prediction, and structural vaccinology, including rational approaches, are more in demand to develop the potential vaccine candidates. Different tools applied for protein scaffolding, and epitope prediction contribute an essential role in vaccine design. This approach is advantageous as it is accomplished by analyzing the entire genome of the pathogen as well as by recognizing the proteins that act as a potential antigens. This allows flexible analysis that cannot be performed by traditional methods. The use of computational tools is beneficial for vaccine researchers, vaccine recipients as well as for public health policy-makers and epidemiologists. These tools may also be used to design vaccines for new, emerging diseases. The development of vaccines requires sound knowledge of immunology along with integration of the different areas, including cell biology, physical chemistry, and computational science. The combination of these disciplines will enhance the discovery of potential vaccine candidates.

Disclosure of potential conflicts of interest

The authors declare no known conflict of interest.

Funding

Authors acknowledge M.D. University, Rohtak, India, for providing infrastructural facilities. SV acknowledges the support as University Research Scholarship by M.D. University, Rohtak, India. The authors wish to acknowledge NIH fellows editorial board for the scientific editing of the manuscript. The authors also acknowledge the infrastructural support from Department of Science and Technology, New Delhi, Govt. of India, through FIST grant (Grant No. 1196 SR/FST/LS-I/ 2017/4).

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