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Immunoinformatics and reverse vaccinomic approaches for effective design

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16.1 Introduction

New Coronavirus pneumonia was discovered in Wuhan, China, in December 2019, and was found initially related to animal-to-human transmission in local wet markets. Following that, human-to-human transmission of the virus began, resulting in widespread respiratory disease in Wuhan and other Chinese cities. After that, the coronavirus spread throughout China and approximately 20 other countries (Li et al., 2020). Furthermore, severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) is highly transmittable, with an estimated reproductive number (R0) of 2.2, implying that one infected person may infect 2.2 others, and with a typical incubation time of 5.8 days. The fact that SARS-CoV-2 can be transferred from asymptomatic infected people (Rothe et al., 2020), as well as its capacity to produce pandemic illness in a matter of weeks, implies that controlling this viral infection will be very difficult without a vaccine. This led to an urge to design a vaccine against the virus as soon as possible to outshine the counterparts of such a dreadful virus. The discipline of pharmacogenomics and pharmacogenetics has lately been dubbed "vaccinomics," which combines pharmacogenomics and pharmacogenetics with bioinformatics-based computational approaches to vaccines design and production (Poland et al., 2009). The massive amount of data generated by whole-genome sequencing projects, combined with the data generated from bioinformatics tools, has revolutionized the field of vaccine research and development, resulting in the development of a "third-generation" vaccine based on the application of vaccinomics science to vaccinology

(Kanampalliwar, 2020). Reverse vaccinology is the first example of such a technique. Reverse vaccinology cuts the time for vaccine development and evaluatation of its efficacy againsttargets. This method looks at the genome and predicts which antigens are most likely to become vaccination candidates (Bagnoli et al., 2011). This outlook enables not only the detection of all antigens discovered previously but also the development of novel antigens that function in a completely different scenario. As a result, this approach aids in the emergence of new immune intervention pathways. Following the Coronavirus disease-2019 (COVID-19) pandemic, immunologists have turned to computational vaccinology and immune-informatics to discover possible immune epitope targets in the SARS-CoV-2 viral genome and variations' classified and unclassified genes. (Dong et al., 2020; Khan et al., 2021) Diverse SARS-CoV-2 vaccine types are now being developed in response to the urgent need to combat COVID-19 which including inactivated vaccines, nucleic acid vaccines, adenovirus-based vector vaccines, and recombinant subunit vaccines (Lu, 2020; Parihar et al., 2020). Some vaccines which have been developed to combat SARS−CoV-2 are enlisted in Table 16.1 which has been extracted from https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/ (COVID- 19 Vaccine Tracker, 2021).

The SARS-CoV-2 has structural and nonstructural proteins via which medication or antiviral agents may interact. Nonstructural proteins (nsps) include 3C-like protease (3CLpro), papain-like protease (PLpro), and other nsps. Structural proteins include spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. (Nittari et al., 2020; Vankadari, 2020). The essential step for SARS-CoV-2 entry into host cells is the attachment of the S protein to the host-cell receptor angiotensin-converting enzyme 2 (ACE2) (Matías-Guiu et al., 2020). In addition, the interaction of the S protein with the cellular transmembrane serine protease (TMPRSS2) may aid viral entry into host cells. As a consequence, pharmacological targets such as ACE2 and TMPRSS2 might be utilized to prevent SARS-CoV-2 infection (Elhusseiny et al., 2020). Various in silico techniques can be utilized to identify unique epitopes from structural proteins such as nucleocapsid, membrane, envelope, and spike (S) protein and nsp. The peptide sequences of these epitopes function as immunological epitopes for TLRs, T-cells, and B-cells (Tahir Ul Qamar et al., 2021). Immunoinformatics is the study for host-pathogen interactions and effective vaccine development, as opposed to traditional immunological investigations, which can take years. This approach is affordable, and it leads to a greater knowledge of illness etiology, diagnosis, and immune response (Mirzaei et al., 2020). In the present chapter, vaccine development using immunoinformatics and reverse vaccinology methods has been discussed along with challenges and future aspects.

16.1.1 Immunoinformatics and reverse vaccinomic approaches

Vaccination has unquestionably aided in the promotion of a healthy worldwide population. It has saved lives, lowered healthcare expenses, improved man's quality of life, and is currently being used for the prevention of various infectious diseases (Terry et al., 2014). It has a significant impact on lowering the disease burden, disability, and death in several countries. However, vaccine development is a complicated process and it gets further tedious for newly emerging and re-emerging infectious diseases (ERIDs), infectious agents

TABLE 16.1 The vaccines which are under clinical trials to combat severe acute respiratory syndrome-Coronavirus-2.

S. No.	Name of vaccine	Type of vaccine	Phase	Developer	Dose	Design	Location
1	Medigen MVC-COV1901	Protein subunit	Phase II	Medigen Vaccine Biologics Corporation/NAID/Dynavax	2	Double-blind	Taiwan, Vietnam
2	Vector Institute EpiVacCorona		Phase III	Vector Institute (peptide)	2	Double-blind	Russia
3	Sanofi/GSK CoV2 preS dTM		Phase II	Sanofi Pasteur/GSK	2	Double-blind	USA, Honduras
4	Biological E Ltd BECOV		Phase I/II	Biological E Ltd	2 or 3	Open-label	India
5	Novavax NVX-CoV2373		Phase I/II	Novavax	2	Observer-blind	Japan
6	CureVac CVnCoV	RNA	Phase II/III	CureVac	2	Observer-blind	Argentina, Belgium, Colombia, others
7	Moderna mRNA-1273		Phase III	Moderna/NIAID	2	Observer-blind	USA
8	BioNTech BNT162 (b1/b2)		Phase II	BioNTech/Pfizer/Fosun Pharma	2	Observer-blind	China
9	Arcturus ARCT-021		Phase II	Arcturus/Duke-NUS	1 or 2	Observer-blind, dose-ranging	USA, Singapore
10	Daiichi-Sankyo DS-5670a		Phase I/II	University of Tokyo/Daiichi-Sankyo	2	Double-blind, dose-ranging	Japan
11	Zydus Cadila ZyCoV-D	DNA	Phase I/II	Zydus Cadila Healthcare Limited (DNA)	3	Double-blind	India
12	AnGes AG0302-COVID-19		Phase II/III	Osaka University/AnGes/Takara Bio	2	Double-blind	Japan

(*Continued*)

TABLE 16.1 (Continued)

| S. No.	Name of vaccine	Type of vaccine	Phase	Developer	Dose	Design	Location
13	Inovio INO-4800		Phase II/III	Inovio Pharmaceuticals/International Vaccine Institute	2	Double-blind, dose-ranging	USA
14	OncoSec CORVax12		Phase I	OncoSec Medical Inc/Providence Cancer Institute	2	Open-label	USA
15	Genexine GX-19N		Phase I	Genexine Consortium	2	Open-label	Republic of Korea
16	Symvivo bacTRL-Spike		Phase I	Symvivo	1	Observer-blind, dose-ranging	Australia
17	Sinovac CoronaVac	Inactivated	Phase IV	Sinovac	2	Stepped-wedge clusters	Brazil
18	Bharat Covaxin		Phase III	Bharat Biotech/ICMR/National Institute of Virology	2	Double-blind	India
19	Sinovac CoronaVac		Phase III	Sinovac	2	Double-blind	Brazil
20	WIBP/BIBP vaccines		Phase III	Beijing Institute of Biological Products/Wuhan Institute of Biological Products/Sinopharm	2	Double-blind	Peru
21	VLA2001/ChAdOx1-S		Phase III	Valneva/Dynavax/University of Oxford/AstraZeneca	2	Observer-blind	UK
22	Janssen Ad26.COV2.S	Vector (nonreplicating)	Phase III	Janssen Pharmaceutical Companies	1	Double-blind	USA, Argentina, Brazil, others
23	Gamaleya Gam-COVID-Vac/Sputnik V		Phase III	Gamaleya Research Institute	2	Double-blind	Russia
24	Oxford ChAdOx1-S		Phase III	University of Oxford/AstraZeneca	2	Double-blind	USA, Argentina, Chile, others

(*Continued*)

TABLE 16.1 (Continued)

S. No.	Name of vaccine	Type of vaccine	Phase	Developer	Dose	Design	Location
25	Janssen Ad26.COV2.S		Phase III	Janssen Pharmaceutical Companies	2	Double-blind	USA, Belgium, Brazil, others
26	Oxford ChAdOx1-S		Phase II/III	University of Oxford/AstraZeneca	1 or 2 + /- boost	Single-blind, dose-ranging	UK
27	ReiThera GRAd-COV2		Phase II/III	ReiThera/Leukocare/Univercells	1 or 2	Observer-blind, dose-ranging	Italy
28	Oxford ChAdOx1-S		Phase I/II	University of Oxford/AstraZeneca	1 or 2	Double-blind	South Africa
29	Gamaleya Gam-COVID-Vac/ Sputnik V		Phase II/III	Gamaleya Research Institute	2	Double-blind	India
30	Oxford ChAdOx1-S		Phase I/II	University of Oxford/AstraZeneca	Up to 3	Single-blind, dose-ranging	UK

with complex lifecycles and antigenic diversity, and the requirement for a tailored vaccine candidate (Poland et al., 2016; Servín-Blanco et al., 2016). Many infectious agents genomes are known particularly those that are new or have antigenic diversity, but their immunological consequences of protection are still unknown. Some of these factors contribute to the difficulty of developing vaccines for ERID and multilifecycle pathogenic illnesses (Servín-Blanco et al., 2016; Terry et al., 2014). The advent of a novel pattern of vaccine design has emerged from serendipitous immunology findings combined with knowledge of bioinformatics techniques for epitope predictions. In immunology research, the art and science of fast and comprehensive information extraction and analysis of data published in relevant databases are becoming increasingly important (Manzoni et al., 2018). Despite this capability (efficient information extraction), several obstacles in the application of bioinformatics in immunology include structure and/or function analysis, as well as immune process assessments as they relate to immune interaction specificity. Fortunately, despite the fact that immunology research is both expensive and time-consuming, massive amounts of data are frequently created (Peng et al., 2019). Bioinformatics tools provide the easiest way to analyze such data with high precision and speed. Genome sequencing and in vitro T-cell validation, for example, can be completed in a matter of months rather than years as with traditional vaccine design (Peng et al., 2019). Additionally, computational immunological approaches dramatically cut epitopes screening time and manpower requirements. By examining the protein sequences of a pathogen of interest with computational immunology tools, vaccine candidate epitopes can be discovered. Many of these proteins have not yet been identified or cloned (Urrutia-Baca et al., 2019).

Immunology research generates massive amounts of data. Furthermore, with proteomics and genomes initiatives, as well as intensive pathogen screening and/or pathogen-host interaction, it has become increasingly vital to collect, manage, and analyze this data, resulting in the emergence of immunoinformatics. Immunoinformatics is the study of immunological processes using computational approaches and resources (Zeya llah et al., 2021). Immunoinformatics employs statistical, computational, mathematical, and biological knowledge and techniques to properly and precisely store and evaluate data pertaining to the immune system and its operations. To deal with evidentiary diversity, immunoinformatics employs techniques that span numerous domains of bioinformatics, including database building and maintenance, the use and description of structural and functional signatures, and the development and implementation of predictive tools (Hegde et al., 2018; Mills et al., 2015). These tactics can work together to get a better understanding of the immune systems of both humans and animals, as well as to combat some less anticipated diseases. The complex architecture of vertebrates' immune systems, the changeable nature of infections and environmental antigens, and the multiregulatory pathways indicate that massive amounts of data will be required to reveal how the human immune system works. Conventionally, much cannot be accomplished due to the complexity of the immune system and the virulent antigen, but with the application of computational vaccinology, vaccine design research has become easier, more precise, and more specific (Zeyaullah et al., 2021). The current strategy employed for state-of-the-art immunoinformatics based creation of a multiepitope-based vaccine developements are presented in Fig. 16.1.
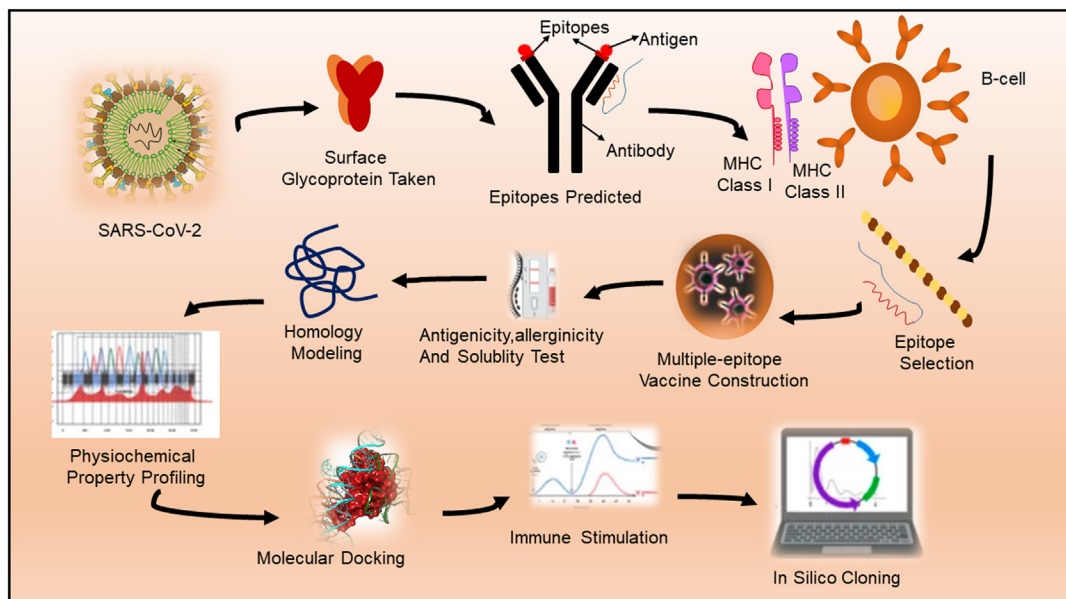
FIGURE 16.1 Various immunoinformatic steps to develop a multiepitope vaccine construct to combat dreadful diseases like severe acute respiratory syndrome-Coronavirus-2.

There are various steps that are put forth in order to design a vaccine using the immunoinformatics approach described in detail in the following subsections.

16.1.1.1 Protein selection and determination of antigenicity /allergenicity

The protein from which epitope has been identified can be selected using various databases and this is the most crucial step for vaccine designing. Researchers have selected structural proteins such as nucleocapsid, membrane, envelope, and spike (S) protein and nonstructural protein of SARS-CoV-2 for epitope extraction. All protein information of SARS-CoV-2 proteomes is available in databases that can be collected. For instance, one can collect information from ViPR (https://www.viprbrc.org/) database. Afterward, the amino acid sequences of the selected proteins can be taken in FASTA format from the NCBI database, available at https://www.ncbi.nlm.nih.gov/ and then analyzed using the BLASTp tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE = Proteins) for similarity search. Exomembrane, physiochemical, antigenic, and allergenic characteristics could be further investigated using online servers such as TMHMM v2.0 (http://www.cbs.dtu.dk/services/TMHMM/), ProtParam (https://web.expasy.org/protparam/), VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and AllergenFP v1.0 (http://ddg-pharmfac.net/AllergenFP/).

16.1.1.2 Screening of helper T-cell lymphocyte

Helper T-cell lymphocyte (HTL) is a crucial part of the adaptive immune system that detects foreign antigens and activates B and cytotoxic T-cells, causing the infectious

pathogen to be destroyed (Xu et al., 2020). Basically, these cells recognized a unique stretch of peptides known as epitopes present on infectious agents. The HTL epitopes can be determined using the IEDB's MHC class II binding allele prediction tool, which is available at http://tools.iedb.org/mhcii/. The HTL epitopes were chosen using the CONSENSUS technique based on a percentile rank of 5% (Wang et al., 2010). The antigenicity and cytokine-inducing capacities of the anticipated epitopes, that is, interferon (IFN) (http://crdd.osdd.net/raghava/ifnepitope/), interleukin-4 (IL4), and interleukin-10 (IL10), can be assessed using various other in silico tools. With default settings, the antigenicity can be predicted using the VaxiJen v2.0 server, while IFN, IL4, and IL10 characteristics can be predicted using the IFNepitope, IL4pred (http://crdd.osdd.net/raghava/il4pred/), and IL10pred (http://crdd.osdd.net/raghava/IL-10pred/) servers, respectively (Dhanda et al., 2013; Nagpal et al., 2017).

16.1.1.3 Screening of cytotoxic T-lymphocyte

Cytotoxic T-lymphocytes (CTLs) are one amongst numerous immune system cells that have the ability to destroy other infectious cells directly (Xu et al., 2020). They immediately enter the viral cell and contribute to the host's defensive system. The sequence of the chosen protein can be used to predict CTL using the NetCTL v1.2 server, which can be accessible at http://www.cbs.dtu.dk/services/NetCTL/, to predict CTLs epitope (Larsen et al., 2007). The VaxiJen v2.0, MHC class I immunogenicity (http://tools.iedb.org/immunogenicity/), ToxinPred (http://crdd.osdd.net/raghava/toxinpred/), and AllerTop v2.0 (https://ddg-pharmfac.net/AllerTOP/) servers can further evaluate the predicted epitopes (Abdelmageed et al., 2020; Dimitrov et al., 2013; Gupta et al., 2013; Doytchinova & Flower, 2007). For all of the forecasts, the servers' default settings can be utilized.

16.1.1.4 Screening of B-cell epitope

B lymphocytes are important arms of the humoral immune response which specifically recognized epitopes on infectious agents. B-cell epitopes can be predicted via the IEDB Ellipro tool (http://tools.iedb.org/ellipro/) with the default settings. Ellipro employs a structure-based approach to anticipate antibody epitopes by calculating the protein's shape, neighboring residues, and protrusion index (PI). The linear B-cell epitopes in the receptor-binding domain of SARS-CoV-2 could be obtained using the ABCpred service to develop a vaccine candidate. It's been discovered that B-cell receptors on B-cells can identify B-cell epitopes. The antigenic areas found on the surface of any pathogen are known as B-cell epitopes. This antigen recognition can activate B-cells, causing them to produce specific antibodies against the antigen. As a result, B-cell epitopes must be taken into account while developing effective vaccinations against the pathogen (Kringelum et al., 2013).

16.1.1.5 Vaccine construct preparation

To be a good subunit vaccine candidate, epitopes that are highly antigenic and simultaneously nonallergic, 100% conserved, overlapping, with significant population coverage, have a strong binding affinity with a common human allele, and have no similarity with human proteins are usually preferred (Hoover et al., 2003). As a result, only those epitopes

that fulfilled the above criteria were chosen to build vaccines against target infectious agents. Further, an adjuvant can also be linked with the epitopes via the EAAAK linker, while the other epitopes could be linked via the AAY and GPGPG linkers to preserve their separate immunogenic activity after their interinteraction compatibility validation (Hoover et al., 2003). The vaccination model is then supposed to run through two separate servers—ProSA-web and PROCHECK—to assess the structural correctness of the model. The Ramachandran plot can also be used to assess the quality of vaccine structure (Laskowski et al., 2006; Wiederstein & Sippl, 2007).

16.1.1.6 Vaccine two-dimensional structure prediction

The PSIPRED 4.0 server (http://bioinf.cs.ucl.ac.uk/psipred/) can be used to evaluate the secondary structural characteristics of the prepared vaccine. This server utilizes two feed-forward neural networks for accurate prediction based on the position-specific score matrix created by PSI-BLAST. The vaccine candidate's solvent accessibility can be predicted using the RaptorX property server (http://raptorx.uchicago.edu/StructurePropertyPred/predict/), which could employ in an emergent machine learning model called DeepCNF viz. Deep Convolutional Neural Fields, for solvent accessibility prediction.

16.1.1.7 Vaccine three-dimensional structure modeling and prediction

Various servers are available for in silico three-dimensional structure modeling of vaccine construct. For instance, the iterative threading modeling approach on the I-TASSER server, available at https://zhanglab.ccmb.med.umich.edu/I-TASSER/ could be utilized to predict tertiary structure for a multiepitope vaccine. Initially, the server searches for appropriate structural templates against the provided protein sequence using Local *Meta-*Threading Server (LOMET). After that, a full atomic structure of the query is created using an iterative template-based fragment assembly simulation. Finally, five complete atomic models of the query sequence are created, each with its own C-score and TM-score. A two-step method using the GalaxyRefine server can further be used to refine the best model. The GalaxyLoop server (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type = LOOP) filtered the loops of vaccine construct using the GalaxyRefine tool, available at http://galaxy.seoklab.org/cgi-bin/submit.cgi?type = REFINE. Following this, by running a molecular dynamics simulation, the GalaxyRefine server rebuilds or re-adjusts the side-chain rotamers of a protein structure, followed by moderate and vigorous relaxing stages to remove conflicts and poor interactions. The Ramachandran plot acquired from the PROCHECK server (https://servicesn.mbi.ucla.edu/PROCHECK/) assesses the stereochemical characteristics of the model vaccine construct. Z-score via ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) can be used to validate the overall quality of the projected structure. Simultaneously, the ERRAT server (https://servicesn.mbi.ucla.edu/ERRAT/) can be used to assess the statistics of nonbonded atom−atom interactions.

16.1.1.8 Physiochemical properties of vaccine and in silico coding

The vaccine constructs physiochemical characteristics can be examined using the online web tool ProtParam (https://www.expasy.org/protparam/), while solubility,

allergenicity, and likely antigenic prediction can be conducted using the online web servers protsol, AllerTOP v. 2.0, and VaxiJen v2.0. The J-CAT tool (http://www.jcat.de/) is helpful to optimize the codons of vaccine constructs. The vaccine design can be cloned in silico by inserting into the pET-28a vector using SnapGene software (https://www.snap-gene.com/try-snapgene/).

16.1.1.9 Immune simulation studies

C-ImmSim examines the status of the cell's consecutive and effective immune responses, and models immune cell memory using a technique that extends their half-life. As a result of this, a small number of cells have a significantly longer half-life and survive longer than other cells. Immunological simulation findings on the ImmSim server are consistent with actual immune responses. Furthermore, a favorable process can be seen as an increase in the B-cell population coupled with an increase in immunoglobulin expression, which resulted in a drop in antigen concentration. In addition, memory development is associated with an increase in Th (helper)-cell population. It is also observed that following vaccination, IFN- γ production level increases. The findings from immune stimulation specifically demonstrated that the T-cell population is extremely sensitive as they are responsible for memory formation, apart from which all other immune cell populations were constant.

16.1.1.10 Immunoinformatics approaches used for vaccine construct for COVID-19

Bioinformatics is an interdisciplinary science that analyses biological data and makes predictions about gene regulatory networks using computational simulation approaches. It's been used effectively in vaccine research, covering preclinical, clinical, and postvaccination phases (Dhama et al., 2020; Ribas-Aparicio et al., 2017; Soria-Guerra et al., 2015). Immunoinformatics is a subfield of bioinformatics that employs computational and statistical methods to analyze and develop immunological data and make predictions about immunity and disease etiology (Fig. 16.2) (Abdelmageed et al., 2020; Dimitrov et al., 2013; Gupta et al., 2013).

Epitope and multiepitope vaccines contain immunogenic amino acid peptides against proteins of the target infectious agent. Predicting possible B- and T-cells epitopes for vaccine design, immune protein analysis, and immunization modeling can be aided by using the previously sequenced COVID-19 genome, computational tools, and searchable databases (Enayatkhani et al., 2020; Ong et al., 2020). Different immunoinformatics studies related to T- and B-cells epitopes identification, analysis and for vaccine designing against SARS-CoV-2 have been well documented in Ahmed et al. (2020), Baruah and Bose (2020), Bhattacharya, Sharma, Patra, Ghosh, Sharma, Patra, Lee, et al. (2020); Bhattacharya, Sharma, Patra, Ghosh, Sharma, Patra, Saha, et al. (2020); Grifoni et al. (2020); Kalita et al. (2020); Kumar et al. (2020); Panda et al. (2020); and Sarkar et al. (2020) (Table 16.2).

There is some preliminary study utilizing bioinformatics towards the creation of a COVID-19 vaccine. SARS-associated coronavirus (SARS-CoV) and SARS-CoV-2 exhibit significant gene sequence similarity and comparable B- and T-cell epitopes, according to Grifoni et al. who employed the IEDB and virus pathogen resource (Grifoni et al., 2020). Immunoinformatics and comparative genomic techniques were utilized in another investigation to evaluate a possible T-cell epitope peptide vaccination that targeted the
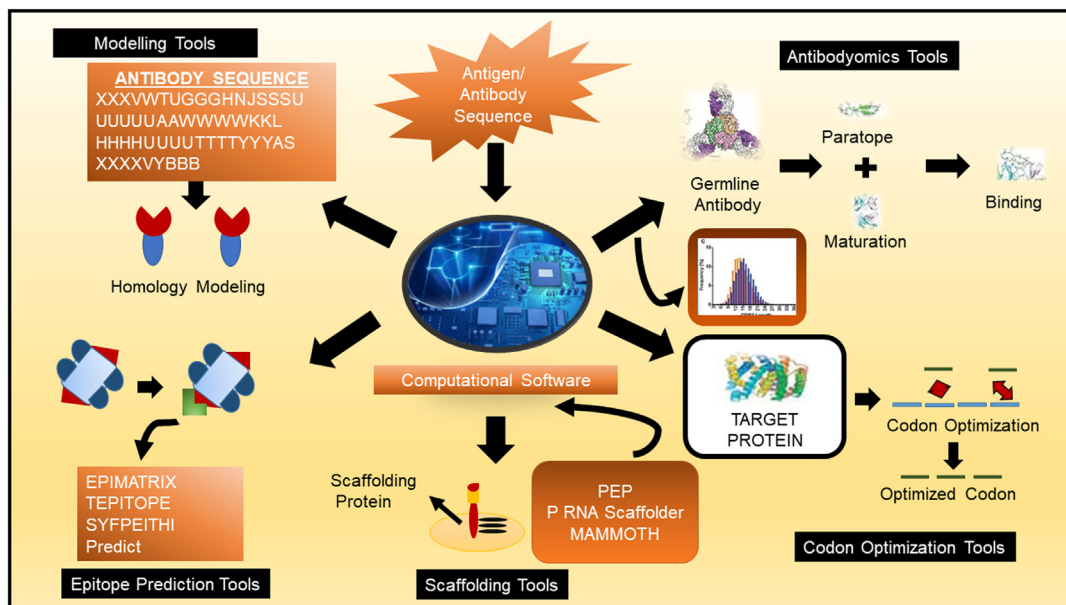
FIGURE 16.2 Computational and statistical methods to analyze and develop immunological data and make predictions about immunity and disease etiology to construct a vaccine against severe acute respiratory syndrome-Coronavirus-2.

COVID-19 envelope protein (CoV-E). Ten MHC Class I and MHC Class II peptides that are potential COVID-19 VCs were discovered using comparative sequencing (Abdelmageed et al., 2020; Dimitrov et al., 2013; Gupta et al., 2013). Enayatkhani et al. also utilized the reverse vaccinomics method to investigate three COVID-19 antigenic proteins [nucleocapsid, ORF3a, and membrane protein (NOM)] and created a possible multiepitope COVID-19 vaccine that stimulates both CD4 + and CD8 + T-cell immune responses (Enayatkhani et al., 2020; Ong et al., 2020). Ong et al. effectively predicted a COVID-19 VC dubbed "Sp/Nsp cocktail" using the Vaxign platform and Vaxign-ML machine-learning technology. SARS-CoV-2, SARS-CoV, and MERS-CoV all have the same protein nsp3 sequence, and the nsp3-domain comprised MHC-I T-cell, MHC-II T-cell, and B-cell epitopes (Ong et al., 2020). Clinical testing and validation of these suggested COVID-19 VCs are the next steps to guarantee effectiveness and safety. Different databases and their web addresses are mentioned in Table 16.3 which includes various immunoinformatics tools and databases to implement reverse vaccinomic approaches.

Using an integrated bioinformatics method, Kwarteng et al. investigated the N-protein of SARS-CoV-2 to discover promising epitope-based vaccination candidates and target the N-terminal region of SARS−CoV-2 N-protein for possible inhibitors. They discovered nontoxic, nonallergenic B-cell and T-cell epitopes that are physically stable, capable of generating IFN-γ, and have a large worldwide population coverage of response. N-protein sequences 404SKQLQQSMSSADS416 and 92RRIRGGDGKMKDL104 have been shown to promote B-cell immunity. They also discovered the T-cell epitopes 79SSPDDQIGY87 and

TABLE 16.2 Vaccine for COVID-19 developed using immunoinformatics approaches.

S. No	Origin	Functions	Proteins	Reference
1	India, South Korea	Common epitopes identification of B-cell and T-cell, antigenicity prediction of T-cell	Spike protein	Bhattacharya, Sharma, Patra, Ghosh, Sharma, Patra, Lee, et al. (2020)
2	India	Epitope identification and antigenicity prediction of T-cell	Spike protein	Kumar et al. (2020)
3	India	Multiepitopic (B-cell and T-cell) peptide identification and antigenicity prediction.	Membrane glycoprotein, Surface spike glycoprotein, Nucleocapsid protein	Kalita et al. (2020)
4	India	Prediction of common epitopes and antigenicity of B-cell and T-cell	Surface glycoprotein	Baruah and Bose (2020)
5	USA	Prediction of common epitopes of B-cell and T-cell.	ORF3a protein, ORF1ab protein, Surface glycoprotein, Nucleocapsid phosphoprotein, a Membrane glycoprotein	Grifoni et al. (2020)
6	Bangladesh	Prediction of common epitopes and antigenicity of B-cell and T-cell.	Surface glycoprotein, Nucleocapsid phosphoprotein	Sarkar et al. (2020)
7	China	Prediction of common epitopes of B-cell and T-cell	Nucleocapsid phosphoprotein, Surface glycoprotein	Ahmed et al. (2020)
8	South Korea, India	In silico cloning and validation of peptide vaccine candidate	Spike protein	Bhattacharya, Sharma, Patra, Ghosh, Sharma, Patra, Saha, et al. (2020)
9	Sweden, India, Denmark	Identification of B-cell and T-cell epitopes	Spike protein and Mpro	Panda et al. (2020)

305AQFAPSASAFFGMSR319, which form stable structures alongside human leukocyte antigens. Relying on docking and simulation research, they have identified zidovudine triphosphate, an anti-HIV drug, as a possible inhibitor of the SARS-CoV-2 N−N-terminal protein's domain, and it should be evaluated for experimental validations. The outcomes of such a study might assist to speed up the development of COVID-19 treatment alternatives (Kwarteng et al., 2020).

In another study, Joshi et al. found an epitope, ITLCFTLKR that is biochemically compatible with HLA allelic proteins. They suggested that this may be utilized as a possible SARS-COV-2 vaccine candidate. Not only do certain putative epitope and HLA-allelic complexes have superior binding scores, but they also have RMSD values in the 0−1 range. According to Ramachandran plot analysis, this epitope has a structural favorability of 99.8%. To reflect further validation of T-cell epitope analysis, an appropriate range of

TABLE 16.3   Software used for in silico vaccine designing using immunoinformatic approaches.

S. No.	Name	Database / immunoinformatic tool	Website URL
1	ArrayPitope	Antigen epitope mapping at the residue level based on peptide microarray data.	http://www.cbs.dtu.dk/services/ArrayPitope/
2	CTLPred	Epitopes of Cytotoxic T Lymphocytes Prediction	http://crdd.osdd.net/raghava/ctlpred/index.html
3	IgPred	Antibody-specific B-cell epitope prediction	http://crdd.osdd.net/raghava/igpred/
4	VaxiJen	Antigen prediction and development of subunit vaccine	http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html
5	JCat	Codon optimization and reverse translation	http://www.jcat.de/
6	PSIPRED	Prediction of the vaccine's secondary structure	http://bioinf.cs.ucl.ac.uk/psipred/
7	Immune Epitope Database (IEDB)	Database of experimentally characterized immune epitopes	https://www.iedb.org/
8	C-ImmSim	Investigate the characteristics of immunogenicity and immune response at the mesoscopic level	http://www.cbs.dtu.dk/services/C-ImmSim-10.1
9	ToxinPred	Toxic/nontoxic peptides can be predicted and designed.	http://crdd.osdd.net/raghava/toxinpred/
10	WebDSV	In silico cloning of vaccine	http://www.molbiotools.com/WebDSV/
11	ProPred	Prediction of MHC class-II binding regions in an antigen sequence	https://webs.iiitd.edu.in/raghava/propred/
12	IMGT®	T-cell receptors, Integrated knowledge resource specialized in the antibodies, MHC	http://www.imgt.org/
13	PDB	Structural database and viewing tools, MHC/peptide/TCR combinations	https://www.rcsb.org/
14	Abcpred	Artificial neural network-based B-cell epitope prediction server	https://webs.iiitd.edu.in/raghava/abcpred/
15	DiscoTope	Prediction of discontinuous B-cell epitopes from protein three-dimensional structures.	http://www.cbs.dtu.dk/services/DiscoTope/

IC50 values and population coverage was acquired. This epitope is well-selected, according to stability analysis using MDWeb and half-life analysis using the ProtParam program. This novel epitope-based vaccination prediction approach is fundamental and simple to use, and it has the potential to be economically useful and practical (Joshi et al., 2020).

Shrivastava et al. used several immunoinformatics and sequence alignment approaches to identify several human B-cell, CD4 + , and CD8 + T-cell epitopes that are highly conserved in over 81,000 SARS-CoV-2 human strains identified to date in 190 countries on six continents; six circulating CoVs that caused previous human outbreaks of the "Common Cold"; and six

circulating CoVs that caused previous human outbreaks of the "Common Cold." Five SL-CoVs from bats, five SL-CoVs from pangolins, three SL-CoVs from Civet Cats, and four MERS strains from camels were discovered. They also discovered cross-reactive asymptomatic epitopes that induced strong B-cell and T-cell responses in "humanized" human leukocyte antigen (HLA)-Dr/HLA-A*02:01 double transgenic mice and recalled B-cell, CD4 + , and CD8 + T-cell responses in both asymptomatic COVID-19 patients and healthy individuals who had never been exposed to SARS-CoV-2. The findings herein pave the way to develop a preemptive multiepitope pan-Coronavirus vaccine to protect against past, current, and potential future outbreaks (Prakash et al., 2020).

Another study was done in which Banerjee et al. used immunoinformatics techniques to create a new multiepitope yet multiprotein vaccine using various proteins of the SARS-CoV-2 that was verified in silico as stable and potential. The inclusion of T-cells along with B-cell inducing epitopes, as well as interferon-gamma generating epitopes in the vaccine, ensures both humoral and cell-mediated immune responses. The final vaccine contains an adjuvant at the N-terminal, as well as epitopes for Cytotoxic T-lymphocytes and Helper T-lymphocytes. The construct was nonallergic and exhibited potential antigenicity. The immune-stimulatory toll-like receptors (TLRs), TLR-2,3,4, were used to molecularly dock the improved, verified tertiary structure model of the vaccine. The binding energies of docked complexes were investigated, and binding interactions between receptor and vaccination were discovered. The vaccine's immunological simulation even verified the beginning of increased host immune responses. An in silico cloning technique was used to demonstrate the vaccine's effective translation into an expression vector. Certainly, the creation of such a vaccine candidate might be a viable COVID-19 treatment (Banerjee et al., 2020).

The spike protein of SARS-CoV-2 was investigated for possible immunogenic epitopes in order to create multiepitope vaccine complexes in research by Naz et al. The S1 and S2 domains of spike proteins were studied into which two vaccine designs with T-cell and B-cell epitopes were prioritized. They have used a comprehensive prediction framework to obtain a new understanding of immunogenic epitopes of spike proteins, which was concluded to be then tested as possible COVID-19 vaccine candidates. Prioritized epitopes were then modeled with linkers and adjuvants, and three-dimensional models were created to assess their physiochemical characteristics and potential interactions with TLR2, ACE2, TLR4, and HLA Superfamily alleles (Naz et al., 2020).

In another study by Rahman et al., the epitopes of the E, M, and S proteins which were antigenic were combined in a multiepitope-based peptide vaccine against SARS-CoV-2 using an immunoinformatics method combined with comparative genomics. Advanced bioinformatics methods were used to predict, improve, and validate the tertiary structure. The proposed vaccination has a global coverage rate of 90.0% for various ethnic groups. The chimeric vaccine's binding to immunological receptors (TLR3 and TLR4) was predicted using molecular docking and dynamics simulations. Immune simulation anticipated a substantial primary immunological response with higher IgM levels and a secondary immune response with high IgG1 and IgG2 levels. Besides, it also boosted the T-helper and cytotoxic T-cell proliferation, as well as the production of IFN- and IL-2 cytokines. The chimera was shown to be appropriate for high-level production and cloning after codon optimization and mRNA secondary structure prediction. Ultimately, the developed recombinant chimeric vaccine candidate showed substantial promise and can be

considered for clinical testing to combat COVID-19, a worldwide concern (Rahman et al., 2020).

An investigation by Singh et al. attempted to use several computational approaches to discover B-cell and T-cell epitopes from the spike surface glycoprotein of SARS-COV-2. They used eight different strains, one each from India, China, France, the United States, Italy, Australia, Iran, and Pakistan. The strain information was taken from the NCBI database. Using different databases and bioinformatics techniques to examine immunological characteristics like surface accessibility, antigenicity, variability, conservancy, flexibility, hydrophilicity, allergenicity, and toxicity of conserved spike glycoprotein sequences. They discovered two possible new linear (SGTNGTKRFDN and ASVYAWNRK) and one structural B-cell epitopes (RLFRKSNLK and IPTNFTISV) as well as two T-cell epitopes (RLFRKSNLK and IPTNFTISV) that can be utilized as epitope-based peptide vaccines. The aforementioned T-cell epitopes had the lowest free binding energy and a strong hydrogen bond interaction in a docking simulation experiment, indicating that they may be used as a T-cell epitope in an epitope-based new vaccine against SARS-CoV-2. With high confidence for the discovered strains, the B-cell and T-cell epitopes highlighted in their paper suggest possible paths for building an exploratory vaccine against the spike surface glycoprotein of SARS-CoV-2.

An effort was made to create an epitope-based vaccine for combating COVID-19 illness by studying the virus's whole proteome using immunoinformatics techniques by Akhtar et al. They obtained the SARS-CoV-2 protein sequence and then tested the proteins for their allergenic potential. After that, they looked for antigenic proteins that could attach to MHC II molecules using nonallergen proteins. The epitopes were then modeled and docked to anticipate their interaction with MHC II molecules. The stability of the epitopes was further investigated using a dynamic stimulation technique. The chosen vaccine candidates were then evaluated for global population coverage against SARS-related coronavirus species. As a consequence, the study predicted five peptide compounds that might be used to build epitope-based vaccines. Because of its high geometric shape complementarity score, low ACE, and extremely strong response by the worldwide population, the peptide LRARSVSPK revealed to be the most powerful epitope among the five chosen epitopes (81.81% global population coverage). The development of a stable epitope-MHCII complex was also revealed by a molecular dynamics simulation study. The LRARSVSPK epitope was also discovered to be substantially conserved among SARS-CoV-2 isolates from various countries. As a result, the study has identified T-cell epitopes that might elicit a strong immunological response in the general public and so serve as prospective vaccination candidates. However, wet lab investigations are needed to confirm these epitopes' capacity to act as vaccine candidates (Akhtar et al., 2020).

In another study a bioinformatic approach to aid in the design of an epitope peptide-based vaccine against the virus's spike protein, mapping out structurally in the spike protein five antigenic B-cell epitopes with viable antigenicity and a total of 27 discontinuous B-cell epitopes for antibody recognition. They found eight CD8 + T-cell 9-mers and 12 CD4 + T-cell 14−15-mers to be interesting candidate epitopes that are putatively limited by a large number of MHC-I and II alleles, respectively. They exploited this knowledge to create an in silico chimeric peptide vaccine with a high translational rate when cloned in the pET28a (+) vector.

When administered as a homologous prime-boost vaccine, the vaccine design was anticipated to elicit high antigenicity and cell-mediated immunity, with the adjuvant linker activating TLR 5. An increase in IgM and IgG, as well as a variety of Th1 and Th2 cytokines, were seen in response to the vaccination. Their immunological simulations showed a reduction in antigen levels after an in silico challenge with SARS-CoV-2. As a result, they've recommended this possible vaccination design as a significant strategy (Chukwudozie et al., 2020).

A study used immunoinformatics to create a new multiepitope vaccine that might possibly trigger immune response via immunogenic and abundantly expressed structural proteins in SARS-CoV-2. Various immunoinformatics methods and databases were used to screen and analyze epitopes. The antigenicity, allergenicity, and population coverage of the vaccine were evaluated. Epitopes were fused together to produce a single vaccine construct (Covax), which was then adjuvanted with 50S ribosomal protein. Covax was tested for physicochemical characteristics, cross-reactivity, antigenicity, and allergenicity. For docking with TLR 4, Covax's tertiary structure was developed, improved, and verified (TLR4). Covax-TLR4 binding affinity was calculated and compared to TLR4-adjuvant as a control. Finally, the immunological response to Covax was simulated and compared to the immune response to adjuvant alone. In Covax 33 epitopes were combined from S (21), E (3), M (5), and N (4) proteins. These include epitopes on the S protein's receptor-binding motif, which is known to be important for viral attachment. Covax was assessed as stable, antigenic, and nonallergenic in a computer simulation. Epitopes were predicted to have a significant global population coverage, particularly in places with high infection rates, indicating that Covax might be effective as a vaccination for the most vulnerable people. Covax may bind to TLR4, indicating possible immunogenicity and excellent characteristics required for an effective vaccination, according to their findings. Overall, the study suggested that this approach could save time, effort, and money in the development of a candidate vaccination against SARS-CoV-2. Covax will be the subject of in vitro and in vivo research (Herrera, 2020).

The antigenic epitopes of the S, M, and E proteins were combined in a multiepitope-based peptide vaccine against SARS-CoV-2 using an immunoinformatics method combined with comparative genomics. Advanced bioinformatics methods were used to predict, improve, and validate the tertiary structure. For diverse ethnic groups, the proposed vaccination demonstrated an average of 90.0% of world population coverage. The chimeric vaccine peptide's molecular docking with immunological receptors (TLR3 and TLR4) predicted effective binding. Immune simulation anticipated a substantial primary immunological response with higher IgM levels and a secondary immune response with high IgG1 and IgG2 levels. It also boosted T-helper and cytotoxic T-cell proliferation, as well as the production of INF- and IL-2 cytokines. The chimera was shown to be appropriate for high-level production and cloning after codon optimization and mRNA secondary structure prediction. Overall, the developed recombinant chimeric vaccine candidate by them showed great promise and can be considered to be used in clinical trials (Rahman et al., 2020).

In a study, the structural proteins of SARS-CoV-2 which is important for its survival and pathogenicity were utilized to predict antigenic epitopes, resulting in the following conclusions. According to their expectations, the antigenic epitopes were capable of inducing a robust humoral as well as cell-mediated immune response. To improve the stability and immunogenicity of the final vaccine, all epitopes were linked together with particular

linkers. The vaccine's physicochemical properties were evaluated. The vaccine's three-dimensional structure was predicted and validated, and it was docked with the human TLR-3 receptor. In addition, in silico molecular dynamics simulations of the vaccine-TLR-3 receptor complex were used to evaluate its dynamic movements and binding stability. They highly recommend manufacturing this vaccine designed by them, which can then be evaluated in vitro and in vivo to determine its efficacy to cure COVID-19 (Kumar et al., n.d.).

The spike protein of SARS-CoV-2 was investigated for possible immunogenic epitopes in order to develop multiepitope vaccine complexes in a research finding. The S1 and S2 domains of spike proteins were studied by them in which two vaccine designs with T-cell and B-cell epitopes were prioritized. They used a comprehensive prediction framework to obtain a new understanding of immunogenic epitopes of spike proteins, which may then be tested as possible COVID-19 vaccine candidates. Prioritized epitopes were then modeled with linkers and adjuvants, and 3D models were created to assess their physiochemical characteristics and potential interactions with ACE2, HLA Superfamily alleles, TLR2, and TLR4 (Naz et al., 2020).

16.1.1.10.1 Challenges and future perspective

The ultimate prophylactic against viral diseases would be considered as an effective vaccination. Historical precedent shows that the development of a vaccine against emerging pathogens would take about 4–28 years. Therefore, the earlier COVID-19 vaccine seems to be accessible in 2036, according to The New York Times, following completion of academic research, a sequence of preclinical and clinical studies also include the construction of factories, production, approval, and distribution. Aside from that, experimental vaccines cannot be injected into people without extensive safety testing, which takes a long time because it involves numerous trial phases with a large number of volunteers of various ages, races, and health conditions, but such trials are critical precursors to the approval of a new vaccine. Besides, the frustration and sorrow, certain recent vaccine candidates that are recruiting volunteers and researchers undertaking many studies have the potential to drastically alter the pandemic situation in the near future. Furthermore, therapeutic medicines that have already been approved to treat various viral infections may be useful in combating COVID-19 if they are shown to be successful in clinical studies. The process of vaccine development, manufacture, and distribution are easier to reach out through existing pharmaceutical supply chains to the marketplace As we can see, the number of publications is steadily increasing on successful candidates.

It might aid in the development of an effective COVID-19 vaccine. Providing the most up-to-date guidelines for the prevention, diagnosis, and treatment of symptomatic instances of COVID-19. Bioinformatics techniques are being used to predict epitopes with greater precision and in less time than traditional methods throughout the world, but additional 8 billion individuals are at risk and might benefit from vaccination. Although Moderna's vaccine appears to be effective and safe so far, no firm has ever produced an RNA vaccine before, let alone at the volume required. This organization claims that it can produce millions of doses of the vaccine each month at a plant that is already set up for another vaccine, and the company is looking for more partners. Doctors, nurses, paramedics, babies, toddlers, and pregnant women would be the first candidates for vaccination

delivery if a vaccine becomes available in the next year. High-risk individuals and adults over the age of 65 are among those who should get the vaccination first.

Furthermore, because coronaviruses attacks the upper respiratory tract, where our immune system is weak, developing a vaccine for them poses numerous problems, because the upper respiratory system is seen as an exterior bodily component Finding a way to neutralize the virus "on the outside" of the body is extremely difficult, and because epithelial cells are infected, strong immune responses are not triggered (Gillim-Ross & Subbarao, 2006). Evidently, producing vaccines against viruses without generating excessive immune responses is challenging. If a vaccination triggers an immune response without hitting the target cells, the outcome might be much worse than the initial viral infection. In general, there are several limitations to vaccine development for various diseases. For the COVID-19 vaccine, severe side effects and limitations in use for immunocompromised individuals, and reversion to a virulent vaccination in the event of failure should also be considered as crucial factors. The requirement for numerous booster injections when utilizing live-attenuated vaccinations as well as the shorter protection period would be another concern in this regard (Bowick & Mcauley, 2011).

16.2 Conclusion

A number of computational methods can be used in this work to uncover possible T- and B-cell epitopes in the S-protein of SARS-CoV-2, which were subsequently stitched into a multiepitope vaccine. The newly developed vaccine possesses the immunodominant characteristics that are needed, as well as large population coverage. Importantly, it should be able to robustly bind to the immunological receptor TLR4 and trigger a robust immune response against SARS-CoV-2 infection. The immunoinformatic-based vaccine candidate might be a good place to start when it comes to creating a vaccine against the COVID-19 etiological agent. In addition, the possible epitopes discovered using the immunoinformatic approach can be utilized in future research for therapeutic design. However, more empirical testing is needed to verify that the constructed vaccine to be an efficient SARS-CoV-2 prophylactic.

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