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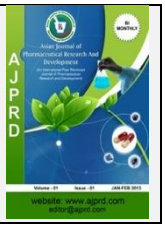

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Review Article

From Code-To-Cure: Harnessing Immunoinformatics for Next-Gen Vaccine Development

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A B S T R A C T

Vaccination is an important intervention in preventing infectious disease epidemics, saving millions of lives, and reducing infection rates.Vaccines have helped treat various illnesses for years by reducing or eliminating disease burdens. The *World Health Organization asserts that three million people are saved yearly because of immunization*. The current direction in vaccine development is towards multi-epitope-based peptide vaccines. Epitope-based peptide vaccines are short protein fragments, known as epitopes, that induce an immune response against a particular pathogen. The conventional approach in vaccine design is labor-intensive, expensive, and time-consuming. Advances in Immunoinformatics and vaccinomics have revolutionized the field of vaccine science, paving the way for next-generation vaccine design. The virtually new constructs of vaccines can be developed by knowledge of Reverse Vaccinology, various repositories of vaccines, and throughput methods. Such *in silico* vaccine research tools are strong, inexpensive, accurate, and safe for humans. The candidates for vaccines have rapidly moved into the stage of clinical trials.The present article will providedetailed information onImmunoinformaticsworking protocol, available databases,and applications of *in silico* vaccine design with recent case studies that will assist researchers in further tailoring vaccines more rapidly and cost-effectively.

KEYWORDS: Vaccine, Multi-epitope, Immunoinformatics, Vaccinomics, *in silico*

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INTRODUCTION

The past three decades have witnessed an unprecedented revolution in our understanding of the immune system, driven by remarkable advances in high-throughput technologies and computational capabilities(1, 2). This convergence of biological discovery and technological innovation has given birth to Immunoinformatics, a discipline that seamlessly integrates computational approaches with traditional immunology(3). As we delve deeper into the intricate mechanisms of immune responses, from molecular interactions to system-wide networks, the necessity for sophisticated computational methods has become increasingly evident, fundamentally transforming how we approach immunological research and clinical applications (4).The genesis of Immunoinformatics can be traced back to the late 1980s when researchers first attempted to predict protein epitopes using rudimentary statistical methods (5, 6). However, the field gained significant momentum during the Human Genome Project, which catalyzed the development of computational tools for biological data analysis (7). This initial spark has evolved into a sophisticated discipline that now encompasses advanced artificial intelligence, complex system modeling, and multi-omics data integration (8). The transformation from simple sequence analysis to comprehensive immune system modeling reflects both technological progress and our growing appreciation of the immune system's complexity, which defies traditional reductionist approaches (9).Modern immunological research generates unprecedented volumes of data through next-generation sequencing, high-throughput proteomics, and single-cell technologies (10). This data deluge presents both opportunities and challenges, necessitating sophisticated computational approaches for

meaningful interpretation (11). Traditional methods of data analysis have proven insufficient for handling the complexity and scale of modern immunological data, making computational approaches not just beneficial but essential for advancing our understanding of immune systems (12).

Contemporary Immunoinformatics has emerged as a multifaceted field, incorporating diverse computational methodologies to address complex immunological questions (13). Machine learning and artificial intelligence now play central roles in predicting immune responses, identifying potential vaccine candidates, and understanding disease mechanisms. The integration of these technologies with traditional immunological research has opened new avenues for understanding disease mechanisms and developing therapeutic strategies (14).The clinical implications of Immunoinformatics extend far beyond basic research. In vaccine development, computational approaches have dramatically accelerated the identification of potential antigens and the optimization of vaccine design (15, 16). In cancer immunotherapy, sophisticated algorithms help predict treatment responses and design personalized therapeutic strategies(17). For autoimmune diseases, computational models provide insights into disease mechanisms and potential therapeutic targets(18).Despite significant progress, the field faces several critical challenges. The integration of heterogeneous data types, computational resource limitations, and the need for improved algorithm accuracy remain ongoing concerns (19). The complexity of immune system interactions, coupled with individual variation and environmental influences, presents formidable challenges for computational modeling(20). However, these challenges also present opportunities for innovation, particularly in the development of more sophisticated AI applications, real-time immune system monitoring, and precision medicine approaches(21).

The systematic approach of computational vaccinology for rational vaccine developmentis illustrated in **Figure 1**. It begins with identifying pathogens of interest, from which potential epitopes are screened using computational tools. These epitopes are then characterized for critical parameters such as allergenicity, antigenicity, and toxicity to ensure safety and effectiveness. Selected epitopes are used to design a vaccine protein sequence, which is subsequently characterized in silico for structural and functional properties. The designed vaccine is then evaluated through protein-vaccine docking studies, followed by immune molecular simulations to predict immune responses, helping refine and optimize the vaccine candidate before experimental validation.

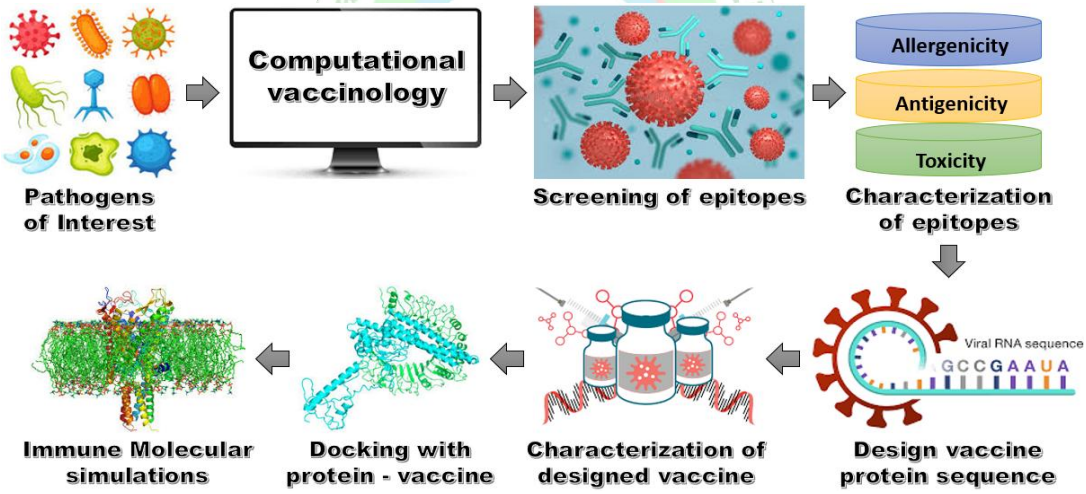

Figure 1: Systematicapproach of computational vaccinology for rational vaccine development

This review aims to provide a comprehensive examination of current Immunoinformatics approaches, their applications, and future directions. Here we explored the core computational methodologies that form the foundation of modern Immunoinformatics and recent successful applications.

Through this study, we seek to provide researcherswith a thorough understanding of how computational tools can be leveraged to advance immunological research.

1. Computational Pipeline for Modern Vaccine Development

The *in-silico* vaccine design or Immunoinformatics has been indispensable for the development of vaccines in terms of antigenicity, safety, and efficacyover the traditional method. The basic schematic epitope vaccine design process is illustrated in **Figure 2**. Traditionally, there are a few methodologies for the design and development of vaccines which been followed over decades. In this outline, the availability of computational resources for the development of clinical-like vaccine candidates are discussed.

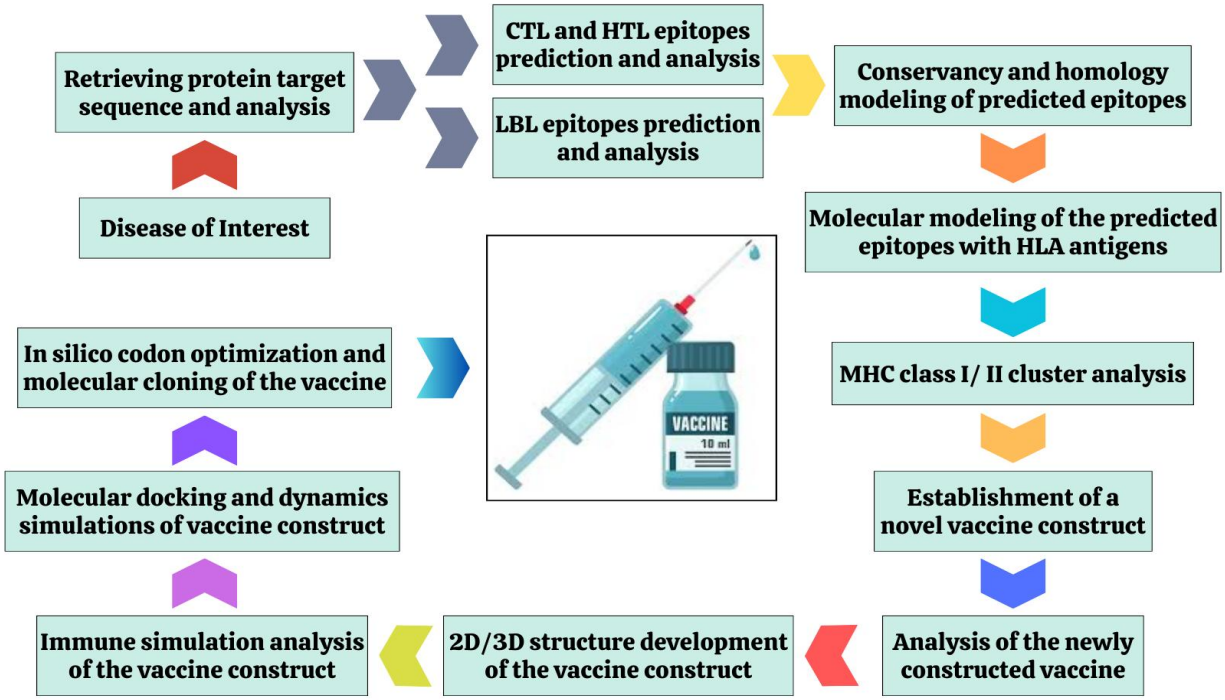

Figure 2: General flowchart of *in silico*vaccine design

Selection and Retrieval of Pathogenic Antigens

The initial phase of computational vaccine development begins with the critical task of identifying and retrieving potential pathogenic antigens. This process leverages sophisticated bioinformatics tools and immunological databases (**Table 1**) to systematically analyze pathogen proteomes for promising vaccine candidates. Researchers utilize comprehensive databases such as the Virulence Factor Database (VFDB) and the Immune Epitope Database (IEDB) to access extensive collections of known virulence factors and immunogenic proteins. Modern computational approaches employ machine learning algorithms that analyze multiple protein characteristics simultaneously, including surface exposure probability, sequence conservation across strains, and potential cross-reactivity with host proteins.Advanced algorithms evaluate protein sequences based on their physicochemical properties, cellular localization, and expression patterns during infection. These tools have revolutionized antigen selection by incorporating evolutionary data, structural predictions, and experimental validation results into their prediction models. Success rates in identifying viable vaccine candidates have improved significantly, with recent studies reporting accuracy rates exceeding 85% when using integrated computational approaches. This marks a substantial improvement over traditional experimental methods, which often require extensive laboratory screening of numerous candidates.

Table 1: Various platforms for protein sequence retrieval

Platforms	References	Webpage
UniProt	(22)	https://www.uniprot.org/
PIR Database	(23)	http://pir.georgetown.edu/
Swiss-Prot	(24)	https://www.expasy.org/resources/uniprotkb-swiss-prot
IMGT	(25)	http://www.imgt.org/IMGTindex/databases.php
EPIMHC	(26)	http://imed.med.ucm.es/epimhc/
JenPep	(27)	http://www.jenner.ac.uk/JenPep
SYFPEITHI	(28)	https://www.syfpeithi.de/
Bcipep	(29)	http://crdd.osdd.net/raghava/bcipep/
kabat database	(30)	http://www.ftp.ebi.ac.uk/pub/database/kabat/

Epitope Prediction

Epitope prediction has emerged as a sophisticated computational challenge in vaccine development, employing various algorithms to identify both B-cell and T-cell epitopes (**Table 2**). Modern prediction tools utilize machine learning approaches, incorporating structural biology data, sequence analysis, and experimental validation results. These methods consider multiple factors simultaneously, including amino acid properties, structural features, and immunological parameters, to predict likely epitope regions with unprecedented accuracy.For B-cell epitope prediction, tools incorporate both linear and conformational epitope analysis, using advanced structural modeling techniques and machine learning algorithms. T-cell epitope prediction focuses on MHC binding affinity, peptide processing, and presentation pathways. Tools like NetMHCpan have achieved remarkable accuracy in predicting MHC-peptide binding, while newer approaches incorporate deep learning architectures to improve prediction reliability. These computational methods consider factors such as peptide length preferences, anchor residue patterns, and MHC allele-specific binding motifs.

Table 2: Resources for B-cell and T-cell epitope predictions

Sr.	Resources	References	Sr.	Resources	References
Linear Bces			**MHC Binders**		
1	Abcpred	(31)	15	IEDB-AR	(32)
2	Bepipred	(33)	16	Propred-I	(34)
3	Lbtope	(35)	17	CTL-Pred	(36)
4	Svmtrip	(37)	18	Netctl	(38)
5	BCPREDS	(39)	19	Mhcpred	(40)
Conformational Bces			20	Netmhcpan	(18)
6	Discotope	(41)	21	RANKPEP	(42)
7	Cbtope	(43)	22	SYFPEITHI	(44)
8	PEPITO	(45)	23	SVMHC	(46)
9	Ellipro	(47)	24	PEPVAC	(48)
10	EPITOPIA	(49)	25	Vaxijen	(50)
11	EPCES	(51)	26	Epidock	(52)
12	SEPPA	(53)	27	Epitop	(54)
13	PEASE	(55)	28	Epijen	(56)
14	Episearch	(57)	29	Netmhc-II	(58)

Epitope Screening and Evaluation

The epitope screening phase involves rigorous computational filtering of predicted epitopes to identify the most promising candidates for vaccine development. This process employs multiple screening criteria, including population coverage analysis, cross-reactivity assessment, and toxicity prediction using various platforms (**Table 3**). Advanced screening methods incorporate molecular dynamics simulations to evaluate epitope stability and accessibility, while machine learning algorithms help prioritize candidates based on multiple parameters simultaneously.Population coverage analysis ensures that selected epitopes will be effective across diverse human populations by considering HLA allele frequencies in different geographic regions. Cross-reactivity screening helps prevent potential autoimmune responses by identifying epitopes that might trigger unwanted immune responses.

Table 3: Resources for epitopeevaluation

Platforms	References	Webpage
VaxiJen	(50)	http://www.ddg-pharmfac.net/vaxijen/
AllerTOP	(59)	http://ddg-pharmfac.net/AllerTOP/
ProtParam	(60)	http://expasy.org/tools/protparam.html
ToxinPred	(61)	http://crdd.osdd.net/raghava/toxinpred/
Protein Sol	(62)	https://protein-sol.manchester.ac.uk/
ProSA-web	(63)	https://prosa.services.came.sbg.ac.at/prosa.php

Design, evaluation, and optimization of vaccine construct

The vaccine design phase integrates selected epitopes into optimal vaccine constructs through sophisticated computational modeling (**Table 4**). This process involves careful consideration of epitope arrangement, linker sequence design, and structural stability prediction. Modern design tools utilize advanced algorithms for codon optimization, ensuring efficient expression in target production systems while maintaining antigenic properties.Structural evaluation employs state-of-the-art protein structure prediction tools like AlphaFold, combined with molecular dynamics simulations to assess construct stability. These analyses consider factors such as solvent accessibility, thermal stability, and potential aggregation tendencies. The design process also optimizes factors affecting manufacturing feasibility, including expression efficiency and purification characteristics.

The final verification phase employs comprehensive computational analyses to ensure vaccine construct functionality and safety. This includes detailed molecular dynamics simulations to verify structural stability under physiological conditions, protein-protein interaction modeling to confirm epitope accessibility, and

immunological synapse simulations to predict vaccine effectiveness. Advanced verification methods incorporate quality metrics such as Ramachandran plot analysis, energy profile assessment, and aggregation tendency prediction. These computational approaches help identify potential issues before experimental testing, significantly reducing development time and costs. The optimization process also considers factors affecting large-scale production, including sequence optimization for expression efficiency and stability under storage conditions. This systematic computational pipeline has dramatically accelerated vaccine development timeframes while improving success rates. The integration of artificial intelligence and machine learning continues to enhance prediction accuracy and development efficiency, making it possible to respond rapidly to emerging pathogens and new disease challenges.

Table 4: Various resources for optimization of vaccine construct

Platforms	References	Webpage
PSIPRED	(64)	http://bioinf.cs.ucl.ac.uk/psipred/
AlphaFold2	(65)	https://alphafold.ebi.ac.uk/
PEP-FOLD3	(66)	http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3
I-TASSER	(67)	http://zhanglab.ccmb.med.umich.edu/I-TASSER/
RaptorX	(68)	http://raptorx.uchicago.edu/
3Drefine	(69)	http://sysbio.rnet.missouri.edu/3Drefine/
GalaxyRefine	(70)	http://galaxy.seoklab.org
SignalP 4.1	(71)	https://services.healthtech.dtu.dk/service.php?SignalP-4.1
TMHMM 2.0	(72)	https://services.healthtech.dtu.dk/service.php?TMHMM-2.0

Molecular docking and dynamics simulations

Computational structural biology has become increasingly important in Immunoinformatics, particularly with recent advances in protein structure prediction. The integration of AlphaFold and similar tools has dramatically improved our ability to model immune system components and their interactions(65).Molecular dynamics simulations have become essential tools for understanding the dynamics of immune system components. These simulations provide detailed insights into peptide-MHC interactions, antibody-antigen binding dynamics, T-cell receptor recognition mechanisms, and Conformational changes in immune proteins. Recent advances in GPU acceleration and distributed computing have made it possible to simulate larger systems for longer periods, providing unprecedented insights into immune system dynamics at the molecular level. The following computational resources, such as ClusPro 2.0(73), HADDOCK 2.4(74), PDBePISA(75), HawkDock(76), GRAMM-x(77), Rosetta, and Hex,are widely utilized for molecular docking and molecular interactions. The docked complexes are further taken for stability, and immune simulation response analysis using GROMACS(78), AMBER(79), CHARMM(80), OpenMM(81), and NAMD(82). The C-ImmSimserver is used for the immune simulation,which works based on a machine-learning-basedscoring matrix to predict immune epitopes to evaluate immune response interactions

2. Recent Applications of Immunoinformatics *In silico* vaccine for human parechovirus

Human parechovirus (HPeV) poses a significant threat to newborns, causing severe encephalitis, meningitis, myocarditis, and sepsis,leading to paralysis(83). So far, no specific clinical therapies are available(84). Hence, there's an urgent need tofulfill these unmet needs. Epitope-based vaccines could be a promising approach that offers high potency and safety by focusing on specific immune responses(85).Recently, Sarker and his co-workers successfullydesigned novel multi-epitope HPeV vaccines using Immunoinformatics(86). Their study analyzed three outer membrane proteins, VP0, VP1, and VP3,using Seaview software from six HPeV strains (Q66578.1, O73556.1, BAC23086.1, ABC41566.1, Q9YID8.1, and BAF63403.1) to create consensus protein sequences. These sequences underwent comprehensive evaluation for antigenicity (using Vaxijen), allergenicity (using AllerTOP), and toxicity(using ToxinPred). They identified key T-cell and B-cell adaptive epitopes from these consensus sequences with MHC-II epitopes using NetMHCII v2.3,which supportedcellular and humoral immune responses and MHC-I epitopes specifically targeting infected cells. Both designed epitopes proved to be non-allergic,non-toxic, and showed good antigenicity with a limit of 0.70 scores using the IEDB tool. The vaccinesHPeV-Vax-1 and HPeV-Vax-2 possess favorable hydrophilic propertieswhich is estimated by GRAVY using ExpasyProtParamshowing values of -0.356 and -0.451, respectively. In addition, the water solubility content scores of 0.99 and 0.98, respectively using Protein-Sol and SOLpro. Further, the team identified key disulfide bondsusing Design 2.13 that formedfive amino acid pairs for HPeV-Vax-1, and three pairs for HPeV-Vax-2with qualified bonding energies less than 2.2 kcal/mol.The three-dimensional modeling and structural stability assist in understandinghow antigens interact with protein receptors. TLR-4 (Toll-like receptor-4) is an immune cell receptor capable of immune cell activation and innate immunity.Their molecular modeling studies demonstrated substantial binding affinities using I-TASSER, SWISS-MODEL, and ProSA tools. Both vaccines HPeV-Vax-1 and HPeV-Vax-2 were revealed to have good binding free energies of -1465.8 kcal/mol and -1595.6 kcal/mol where whereasΔG showed favorable -11.1 kcal/mol and -12.8 kcal/mol, respectively, against the TLR-4 receptor using

ClusProv2.0 software. The molecular dynamics simulation studies for the robust stability confirmation of the two designed vaccines with the TLR-4 receptor using YASARA and AMBER14 software. Their study confirmed stable interactions, with an average RMSD (root mean square deviation) of 4.422Å and 4.269Å, and the Rg (radius of gyration) of 41.015Å and 40.785Å for the respective complexes.The TLR4-vaccine complexes demonstrated higher hydrogen bondsand showedgreater stability, which were further measured through PCA analysis. The designed vaccines can generate antibodies, B-cells, T-cells, macrophages, and cytokines, assuring a potential immunogenic response. For efficacy confirmation, the designed vaccines showed Codon Adaptation Index (CAI) values of 0.544 and 0.604 for HPeV-Vax-1 and HPeV-Vax-2, respectively,indicating good potential for *E.coli*expression.

In silico mRNA vaccinefor pancreatic cancer

Masum et al. conceived mRNA vaccine development through a multi-step process(87). Initially, they identified and analyzed multiple members of the S100 protein family, including S100-A4, S100-A6, S100-A8, S100-A9, and S100-A11, as potential targets for their vaccine design. They employed computational methods to evaluate various epitopes for both cytotoxic T lymphocytes (CTL) and helper T lymphocytes (HTL), ensuring optimal binding with specific HLA alleles using IEDB server, IL-10Pred, and IFNepitope.During the design phase, the team carefully considered both B-cell and T-cell responses. Their analysis revealed that the selected epitopes demonstrated high antigenicity while maintaining minimal allergenicity and toxicity profiles using Vaxijen and ToxinPredservers. The constructed vaccine showed impressive physical characteristics, including a molecular weight of 165023.50 Da, and high solubility using Expasy'sProtParam, SOLpro, and Protein-Sol. The structural analysis yielded favorable results with the Ramachandran plot showing 90.6% of residues in preferred regions, and 7.05% in allowed regions using PSIPRED, GOR4, and SOPMA servers.Further, the team conducted molecular interaction studies to assess the vaccine's effectiveness using the ClusPro 2.0 server. The docking analyses revealed strong binding affinities between the vaccine and immune cell receptors, particularly TLR-2 (PDB ID: 2Z7X), and TLR-4 (PDB ID: 3FXI) showing binding energies of -141.07 kcal/mol and -271.72 kcal/mol, respectively. To optimize production potential, they performed codon optimization for *E. coli* expression, achieving a GC content of 47.04% and an optimal CAI score of 1.0.Immune response simulation studies yielded particularly promising results using the C-ImmSim server. The vaccine demonstrated the ability to generate sustained B-cell and T-cell responses, with memory B-cells showing persistence over one year. Notably, the researchers observed immediate increases in IFN-γ and IL-2 concentrations following initial administration, with levels maintaining elevation upon repeated antigen exposure. This indicated successful activation of T-helper cells and robust humoral immune response generation.The final vaccine construct showed remarkable stability, with a minimum free energy of -1760.00 kcal/mol using the HawkDock, suggesting strong potential for successful cellular uptake and expression.

In silico vaccine for *Echinococcus granulosus*

Researchers conducted a comprehensive study on developing an improved vaccine against*Echinococcus granulosus*, a globally prevalent parasitic disease that particularly impacts developing nations. The study aimed to address the limitations of existing vaccination approaches, including live vaccines, DNA vaccines, and the EG95 vaccine, which have faced various challenges in effectiveness and implementation. Khan et al. 2025 employed a computational vaccinology approach, focusing on immunodominant epitopes in antigenic peptides(88). They specifically targeted the AgB protein, utilizing five of its peptide subunits that are expressed throughout the parasite's life cycle. Their methodology incorporated various bioinformatics tools and servers to design and analyze a multi-epitope vaccine.The developed vaccine construct demonstrated promising physicochemical properties. Analysis revealed a molecular weight of 40.21 kDa, an acidic theoretical pI of 7.41, and high thermotolerance with an aliphatic index of 89.93. The vaccine showed hydrophilic characteristics with a negative GRAVY value of -0.421. Antigenicity testing usingVaxiJen and ANTIGENpro yielded scores of 0.8988 and 0.5892, respectively, indicating strong antigenic potential. Importantly, allergenicity assessments using AllergenFP v1.0 and AllerTOP v2.0 confirmed the vaccine would not trigger allergic reactions.Structural analysis revealed that the vaccine comprised 30.84% alpha helix, 67.91% random coil, and 1.24% extended strand. The researchers used I-TASSER for tertiary structure prediction and further refined it using GalaxyRefine, achieving impressive quality metrics: GDT-HA of 0.9725, RMSD of 0.299, and 90.1% of residues in favorable regions according to Ramachandran plot analysis.The team identified ten immunodominant B-cell epitopes (five each for MHCI and MHCII) through the ElliPro tool on the IEDB server. Molecular docking studies demonstrated strong binding affinity to both HLA-A01.01 (MHC-I) molecules (energy score: 9939.5) and HLADRB1*07:03 (MHC-II) molecules (energy score: 985.1). For practical implementation, they optimized the vaccine for E. coli K12 strain expression, achieving ideal parameters with a CAI of 1.0 and GC content of 52.38%.The successful cloning of the MEV into the pIB2-SEC13-mEGFP(+) vector demonstrated its potential for expression and heterologous production.

In silico subunit vaccine for porcine epidemic diarrhea

A potential research team tackled the critical challenge of Porcine Epidemic Diarrhea (PED), which causes up to 100%

mortality in neonatal pigs, by developing a novel multi-epitope vaccine. They focused on the spike (S) protein of PEDV, targeting both its S1 and S2 domains due to their role in viral entry and host cell fusion.Recently, Sira et al. designed a vaccine to trigger comprehensive immune responses by incorporating multiple epitopes (89). They identified ten CTL epitopes (using NetMHCcons 1.1), and four HTL epitopes (using NetMHCIIpan-4.0)that met their criteria for antigenicity, non-allergenicity, toxicity, and immunogenicity using Vaxigen, ToxinPred, and AllerTOP v2. Additionally, they discovered six linear B-cell epitopes and 13 key residues across 19 B-cell epitopes. These components were strategically linked using specific peptide sequences: "KK," "AAY," and "GPGPG" for enhanced epitope presentation.To improve vaccine efficacy, the researchers incorporated several innovative elements. They conjugated Cholera toxin subunit B (CTB) as an adjuvant and added the homing peptide "CTGKSC" to target M cells for improved mucosal immunity. The final construct, named fMEVc, demonstrated favorable safety profiles, being non-allergenic, non-toxic, and antigenic.Structural analysis using Alphafold2 and molecular docking studies (using Cluspro 2.0) revealed strong binding to TLR4 with an energy of -18.0 kcal/mol. Molecular dynamics simulation (using GROMACS 2023) showed the fMEVc-TLR4 complex stabilized at 75ns, confirming activation potential. Immune simulation testing demonstrated promising results after three immunizations at 7-day intervals, showing increased IgG production and memory B cell induction compared to control groups.The study's outcomes suggested that their vaccine design could effectively induce robust mucosal immune responsesfor protecting neonatal pigs through lactogenic immunity.

CONCLUSION AND FUTURE PROSPECTS

The landscape of vaccine development has been fundamentally transformed by computational approaches, particularly in responding to urgent public health challenges. Traditional vaccine development methods, while effective, are constrained by time-consuming processes and substantial costs. The emergence of computational vaccinology, supported by vaccinomics and Immunoinformatics strategies, has revolutionized how we approach vaccine design, offering more efficient and economical pathways to identify and screen potential antigens. This advancement is particularly crucial in addressing emerging pathogenic threats that require rapid response capabilities.The post-genomic era has ushered in the promising concept of multi-epitope-based peptide vaccines, leveraging the vast availability of microbial genomesand proteome sequences. Through sophisticated bioinformatic tools and algorithms, researchers can now identify top immunogenic protein candidates with unprecedented precision. This approach not only accelerates the vaccine development pipeline but also enables the creation of more targeted and effective vaccine constructs. The integration of computational tools for epitope prediction and protein structure analysis has become instrumental in modern vaccine design, though their success ultimately depends on rigorous validation through both *in vitro* and *in vivo* studies.While epitope-based vaccines present numerous advantages over traditional approaches, including improved specificity and safety profiles, they face certain challenges, particularly regarding immunogenicity. These challenges are being addressed through innovative strategies such as multi-epitope approaches, nano-formulations, and liposomal-delivered mRNA technologies. Combining computational prediction methods with experimental validation represents the optimal pathway for assessing vaccine candidates' potential immunogenicity. This comprehensive approach, incorporating *in-silico* design, *in vitro* testing, and *in vivo* validation, provides a robust framework for developing next-generation vaccines.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for publication.

AUTHORS CONTRIBUTION

A.S, Wrote original draft, scientific illustrated, and edited manuscript; **D.S.**, Data curation, original drafted, reviewed, and edited manuscript; **J.P.**,Conceived idea, supervised, and reviewed the manuscript.

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