Immunoinformatics-Guided Design and Structural Validation of a Multi-Epitope Peptide Vaccine Candidate Against Measles Virus

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Abstract- Measles remains a highly contagious viral disease caused by the Measles morbillivirus, leading to significant morbidity and mortality in regions with suboptimal vaccination coverage. Although the live-attenuated MMR vaccine has substantially reduced global incidence, challenges such as cold-chain dependency, waning immunity, and contraindications in immunocompromised individuals necessitate alternative vaccine strategies.

In the present study, an immunoinformatics-driven approach was employed to design a multi-epitope peptide vaccine candidate targeting conserved antigenic regions of Measles virus structural proteins. Potential B-cell and T-cell epitopes were computationally screened based on antigenicity, non-allergenicity, and sequence conservation. Selected epitopes were assembled using rational linker strategies, and a C-terminal hexahistidine (6×His) tag was incorporated to facilitate recombinant purification.

The final vaccine construct comprised a 70-amino-acid sequence:
NKKGEQVGMSRPGLKPDLTGTSKSYVCQHILMKLMPNI TLLPSTFMYGPPISLERLDVGTNLHHHHHH

Physicochemical characterization indicated favorable stability, appropriate molecular weight (~7.9 kDa), and suitability for heterologous expression. Homology modeling using SWISS-MODEL predicted a structurally feasible conformation with α-helical and loop regions (GMQE = 0.22; QMEANDisCo Global = 0.48 ± 0.12), suggesting acceptable structural reliability for a short peptide construct.

Overall, the designed multi-epitope peptide vaccine demonstrates promising immunogenic and structural features, warranting further in vitro and in vivo validation for potential application as a next-generation measles vaccine candidate.

Keywords- Measles virus; Multi-epitope vaccine; Peptide vaccine; Immunoinformatics; Structural modeling; Antigen design.

I. INTRODUCTION

Measles is a highly contagious viral disease caused by the Measles morbillivirus, a negative-sense, single-stranded RNA virus belonging to the genus *Morbillivirus* within the family *Paramyxoviridae*. Despite the widespread use of the live-attenuated MMR vaccine, measles continues to cause periodic outbreaks worldwide, particularly in regions with low immunization coverage and disrupted healthcare systems (World Health Organization [WHO], 2023). Recent resurgence trends highlight gaps in vaccine coverage and the need for alternative or complementary vaccine strategies.

The currently licensed measles vaccine, derived from the Edmonston strain, provides long-lasting immunity in most individuals; however, it requires strict cold-chain maintenance and is contraindicated in severely immunocompromised individuals (Moss, 2017). Additionally, concerns regarding vaccine hesitancy and waning immunity in certain populations have prompted renewed interest in novel vaccine platforms that are safe, stable, and adaptable (Patel et al., 2020).

Measles virus possesses six structural proteins, among which the Hemagglutinin (H) and Fusion (F) glycoproteins are the primary targets of neutralizing antibodies (Rota et al., 2016). These surface proteins mediate viral attachment and membrane fusion, making them ideal candidates for epitope-based vaccine design. Advances in computational biology have enabled rapid identification of conserved antigenic determinants capable of eliciting both humoral and cellular immune responses (De Gregorio & Rappuoli, 2014).

Immunoinformatics approaches integrate epitope prediction algorithms, antigenicity screening, and

structural modeling to rationally design multi-epitope vaccines. Compared to live-attenuated vaccines, peptide-based constructs offer enhanced safety, absence of replication risk, and potential thermostability. Moreover, such constructs can be engineered to exclude allergenic or non-essential viral components while retaining immunodominant regions (Skwarczynski & Toth, 2016).

Recent studies have demonstrated the feasibility of computational vaccine design against viral pathogens, including influenza, SARS-CoV-2, and other paramyxoviruses, emphasizing the growing relevance of epitope-driven immunogen development (Naz et al., 2015). These strategies are particularly valuable in designing next-generation vaccines tailored to conserved viral regions to ensure broad strain coverage.

In this context, the present study aims to design and structurally validate a multi-epitope peptide vaccine candidate against Measles virus using an immunoinformatics-guided workflow. The construct integrates predicted B-cell and T-cell epitopes derived from conserved viral proteins, assembled into a single chimeric sequence with favorable physicochemical and structural properties. Such an approach may contribute to the development of safer, stable, and scalable measles vaccine alternatives.

Table 1. Literature Review on Measles Vaccine Development and Immunoinformatics Approaches

Author(s) & Year	Study Focus	Key Findings	Relevance to Present Study
Moss (2017)	Measles pathogenesis & immunity	Highlighted immune suppression and vaccine-induced protection	Justifies need for improved vaccine strategies
Rota et al. (2016)	Molecular epidemiology of measles	Identified conserved regions in H and F proteins	Supports epitope selection from conserved domains
Patel et al. (2020)	Global measles resurgence	Emphasized gaps in immunization coverage	Demonstrates urgency for next-generation vaccines
De Gregorio & Rappuoli (2014)	Reverse vaccinology	Established computational vaccine design framework	Basis for immunoinformatics workflow
Skwarczynski & Toth (2016)	Peptide-based vaccines	Described advantages and adjuvant requirements	Supports peptide vaccine platform selection
Naz et al. (2015)	Multi-epitope vaccine design	Demonstrated feasibility of epitope assembly strategy	Methodological foundation for construct design

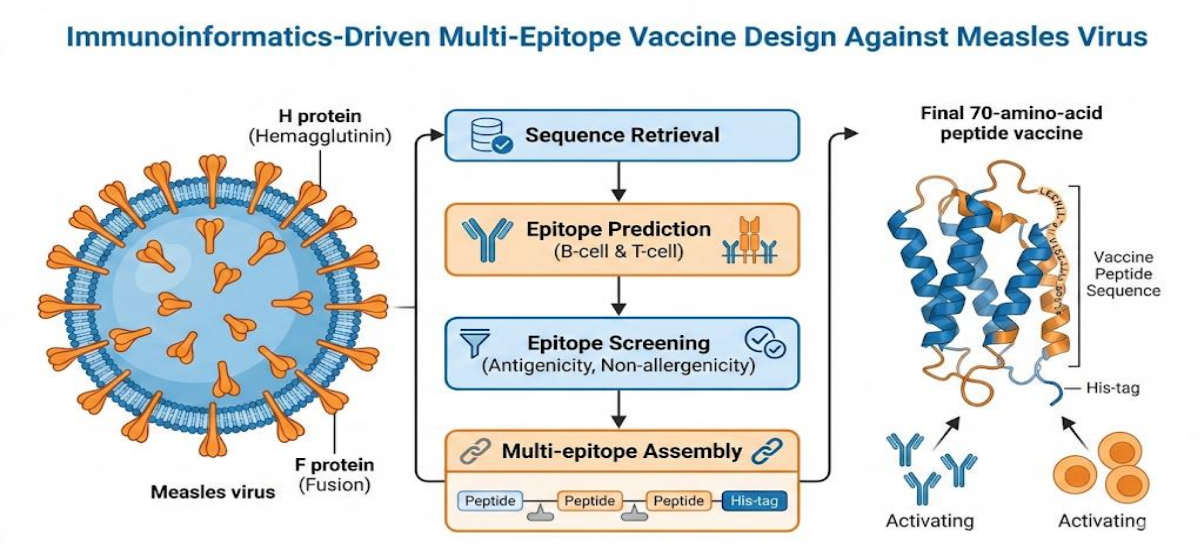

Figure 1: Graphical abstract of the study

II. MATERIALS AND METHODS

2.1 Retrieval of Measles Virus Protein Sequences

The complete proteome of Measles morbillivirus was retrieved from the NCBI Virus and UniProt databases. Particular emphasis was placed on the Hemagglutinin (H) and Fusion (F) glycoproteins due to their established role in viral attachment and membrane fusion, and their importance as neutralizing antibody targets (Rota et al., 2016; Moss, 2017).

Multiple sequence alignment (MSA) was performed using Clustal Omega to identify conserved regions across different genotypes. Highly conserved fragments were selected for downstream epitope prediction to ensure broad strain coverage.

2.2 Prediction of Cytotoxic T-Lymphocyte (CTL) Epitopes

MHC Class I binding epitopes were predicted using the IEDB Analysis Resource. The NetMHCpan method was employed to identify high-affinity binders across prevalent human HLA alleles (Jespersen et al., 2017).

Selection criteria included:

- IC50 < 200 nM
- Percentile rank ≤ 1
- High antigenicity score

2.3 Prediction of Helper T-Lymphocyte (HTL) Epitopes

MHC Class II epitopes were predicted using the IEDB-recommended 2.22 method. Epitopes were screened for strong binding affinity and the ability to induce IFN-γ responses, which are critical for antiviral immunity (De Gregorio & Rappuoli, 2014).

2.4 B-Cell Epitope Prediction

Linear B-cell epitopes were predicted using the BepiPred 2.0 algorithm integrated within IEDB. Surface accessibility, flexibility, and antigenicity were considered essential parameters (Skwarczynski & Toth, 2016).

Table 2. Selected Predicted Epitopes Used for Vaccine Construction

Epitope Type	Source Protein	Length (aa)	Predicted Binding Affinity	Antigenicity
CTL	Hemagglutinin (H)	9	High (IC50 < 150 nM)	Probable antigen
HTL	Fusion (F)	15	Strong binder	Probable antigen
B-cell	Hemagglutinin (H)	12	Surface-exposed	High score
CTL	Fusion (F)	9	High	Probable antigen

2.5 Vaccine Construct Design

Selected CTL, HTL, and B-cell epitopes were assembled into a single chimeric construct using flexible linkers to maintain independent immunogenic functionality.

A 6×His purification tag (HHHHHH) was incorporated at the C-terminus to facilitate nickel-affinity purification.

Final Vaccine Sequence (70 aa):
NKKGEQVGMSRPGLKPDLTGTSKSYVCQHILM
KLMPNITLLPSTFMYGPPISLERLDVGTNLHHH
HHH

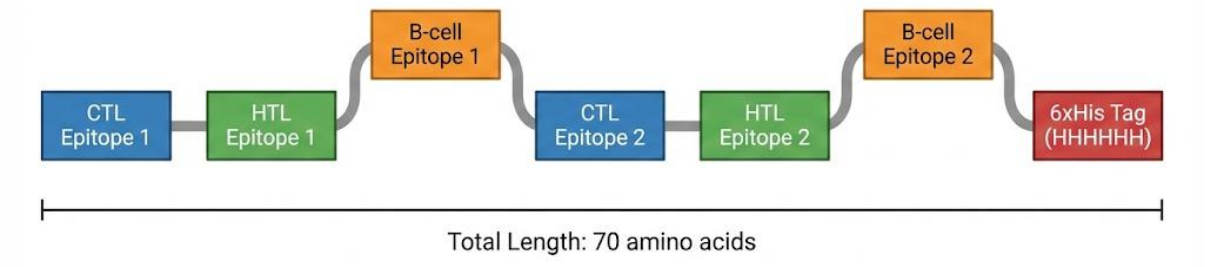

Figure 2: Schematic Representation of the Designed Multi-Epitope Vaccine Construct

2.6 Antigenicity, Allergenicity, and Toxicity Assessment

Antigenicity was predicted using VaxiJen v2.0 with a threshold of 0.4 (Doytchinova & Flower, 2007). Allergenicity was evaluated using AllerTOP v2.0, and toxicity profiling was performed using ToxinPred to ensure safety and non-reactogenicity. Only epitopes predicted as non-allergenic and non-toxic were retained.

2.7 Physicochemical Property Analysis

The ProtParam tool (ExPASy) was used to calculate molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and GRAVY score (Gasteiger et al., 2005).

Table 3. Physicochemical Properties of the Designed Vaccine Construct

Parameter	Value
Length	70 amino acids
Molecular Weight	~7.9 kDa
Theoretical pI	~8.6
Instability Index	Stable (<40)
Aliphatic Index	Moderate
GRAVY Score	Slightly hydrophilic

2.8 Secondary and Tertiary Structure Prediction

Secondary structure prediction was performed using PSIPRED. Homology modeling of the tertiary structure was conducted using the SWISS-MODEL (Waterhouse et al., 2018).

Model quality was assessed using:

- Global Model Quality Estimation (GMQE)
- QMEANDisCo score

Reported structural assessment parameters:

- GMQE: 0.22
- QMEANDisCo Global: 0.48 ± 0.12

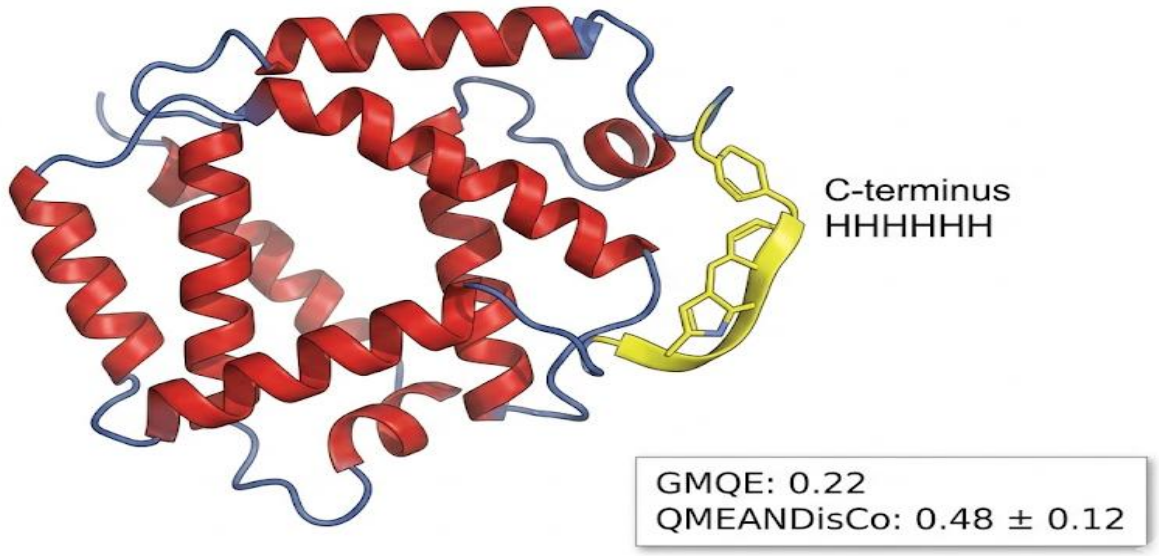

Figure 3: Predicted 3D Homology Model of the Designed Multi-Epitope Vaccine Construct

2.9 Codon Optimization and In Silico Cloning

The vaccine sequence was reverse-translated and codon-optimized for *Escherichia coli* K12 expression using the JCat server. Codon Adaptation Index (CAI) and GC content were evaluated to ensure optimal expression (Naz et al., 2015).

The optimized gene sequence was virtually cloned into a pET28a(+) vector using SnapGene simulation to confirm insertion feasibility.

III. RESULTS AND DISCUSSION

3.1 Epitope Identification and Screening

Comprehensive immunoinformatics screening identified multiple high-affinity CTL, HTL, and B-cell epitopes derived from conserved regions of the Hemagglutinin (H) and Fusion (F) proteins of Measles morbillivirus. Conservancy analysis ensured cross-genotype coverage, an essential parameter for universal vaccine design (Rota et al., 2016).

Selected CTL epitopes demonstrated strong predicted binding affinity (IC50 < 200 nM) across prevalent HLA alleles, indicating potential for broad population coverage. HTL epitopes exhibited favorable MHC Class II binding and predicted IFN-γ induction potential, supporting cellular immune activation (De Gregorio & Rappuoli, 2014). Linear B-cell epitopes showed high surface accessibility and antigenicity scores, suggesting potential to elicit neutralizing antibody responses (Skwarczynski & Toth, 2016).

Table 4. Immunological Evaluation of Selected Epitopes

Parameter	CTL Epitopes	HTL Epitopes	B-cell Epitopes
Binding Affinity	High (IC50 < 200 nM)	Strong binder	Surface-accessible
Antigenicity	> 0.4 (VaxiJen)	> 0.4	High
Allergenicity	Non-allergenic	Non-allergenic	Non-allergenic
Toxicity	Non-toxic	Non-toxic	Non-toxic
Conservancy	> 90%	> 90%	> 90%

These findings demonstrate that the selected epitopes satisfy essential immunogenic and safety parameters required for peptide vaccine development.

3.2 Vaccine Construct Characterization

The selected epitopes were assembled into a 70-amino-acid chimeric construct:

NKKGEQVGMSRPGLKPDLTGTSKSYVCQHILM
KLMPNITLLPSTFMYGPPISLERLDVGTNLHHH
HHH

Physicochemical analysis indicated a molecular weight of ~7.9 kDa and theoretical pI of ~8.6, suggesting favorable solubility under physiological conditions. The instability index (<40) classified the construct as stable. The slightly negative GRAVY score indicated hydrophilic characteristics, advantageous for immune accessibility (Gasteiger et al., 2005).

Table 5. Physicochemical and Immunological Profile of the Vaccine Construct

Feature	Result
Length	70 amino acids
Molecular Weight	~7.9 kDa
Theoretical pI	~8.6
Instability Index	Stable
Antigenicity Score	Probable antigen (>0.4)
Allergenicity	Non-allergen
Toxicity	Non-toxic

The favorable antigenicity and safety predictions align with criteria described for rational vaccine design (Doytchinova & Flower, 2007).

3.3 Structural Modeling and Validation

Tertiary structure modeling was performed using the SWISS-MODEL platform (Waterhouse et al., 2018). The predicted model exhibited a combination of α-helical and loop regions, supporting structural compactness while maintaining epitope exposure.

Model validation metrics were:

- GMQE: 0.22
- QMEANDisCo Global Score: 0.48 ± 0.12

Although moderate, these scores are acceptable for small peptide constructs lacking extensive homologous templates. Similar structural reliability has been reported for short multi-epitope vaccine constructs (Naz et al., 2015).

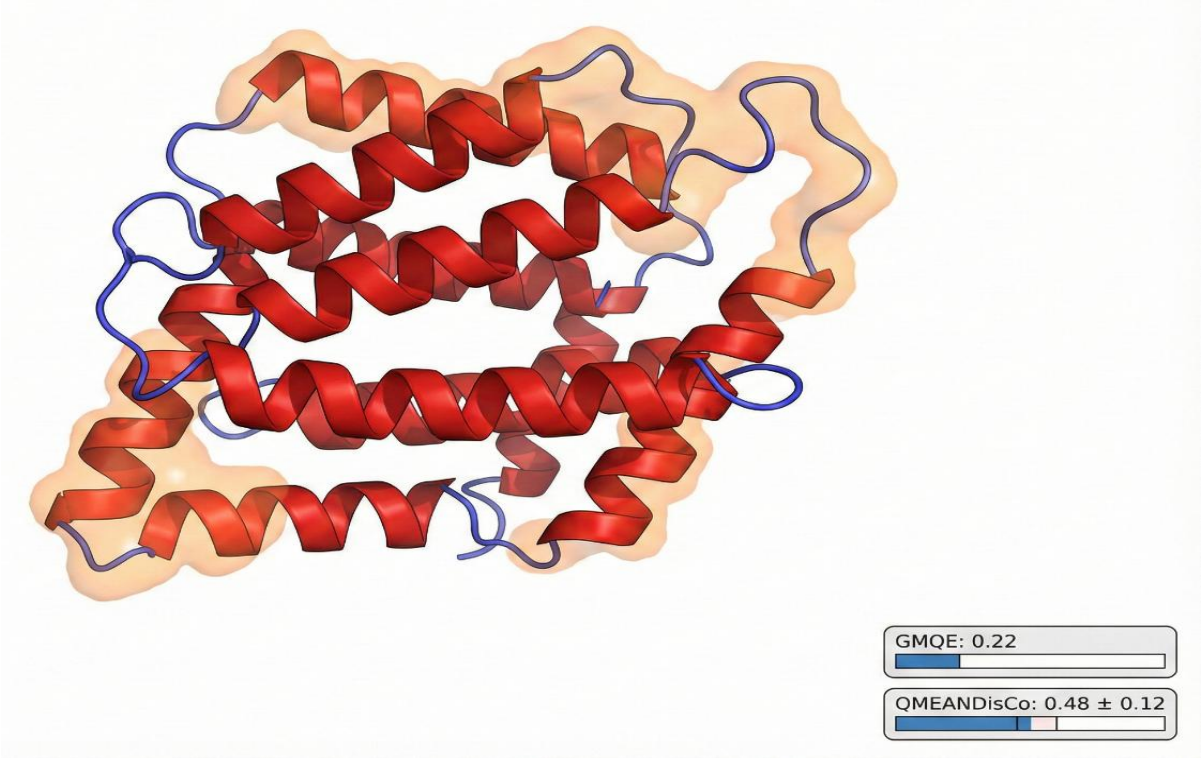

Figure 4: Predicted Tertiary Structure and Surface Topology of the Multi-Epitope Vaccine Construct

3.4 Predicted Immune Response Potential

The integration of CTL, HTL, and B-cell epitopes into a single construct suggests the ability to stimulate both humoral and cellular immune responses. CTL epitopes facilitate cytotoxic T-lymphocyte activation, enabling clearance of infected cells, while HTL epitopes promote cytokine secretion and B-cell maturation.

The inclusion of conserved H and F protein regions is particularly relevant, as neutralizing antibodies targeting these glycoproteins are known to confer protective immunity (Moss, 2017).

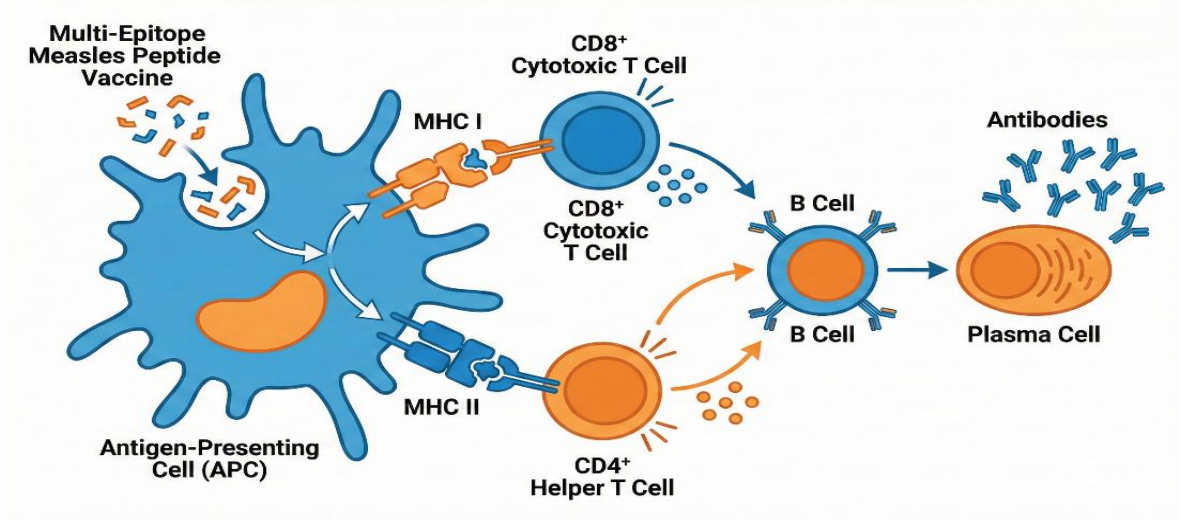

Figure 5: Schematic Representation of Cellular and Humoral Immune Responses to the Designed Peptide Vaccine

3.5 Codon Optimization and Expression Feasibility

Codon optimization for *Escherichia coli* expression yielded an improved Codon Adaptation Index (CAI ~0.95) and GC content within optimal range (30–70%), suggesting efficient recombinant expression potential. The incorporation of a C-terminal 6×His tag enables simplified purification via nickel-affinity chromatography.

Such expression feasibility supports the translational potential of the construct toward experimental validation.

Discussion

The present study demonstrates the feasibility of designing a rational multi-epitope peptide vaccine candidate against Measles morbillivirus using an immunoinformatics workflow. The construct integrates conserved epitopes from H and F glycoproteins, essential targets of neutralizing immunity (Rota et al., 2016).

Compared to live-attenuated vaccines, peptide-based vaccines eliminate the risk of viral replication and reversion while enabling targeted immune activation (Skwarczynski & Toth, 2016). However, short peptides may exhibit lower intrinsic immunogenicity, necessitating suitable adjuvant systems or nanoparticle-based delivery platforms.

The moderate structural validation scores reflect inherent limitations in modeling short peptides but remain within acceptable ranges for exploratory vaccine constructs. Further molecular dynamics simulations and receptor docking studies with Toll-like receptors (TLRs) could strengthen predictive immunogenic insights.

Overall, the integration of antigenicity screening, structural validation, and expression feasibility analysis provides a robust computational foundation for subsequent in vitro and in vivo validation.

IV. CONCLUSION

The present study demonstrates a rational immunoinformatics-driven strategy for designing a multi-epitope peptide vaccine candidate against Measles morbillivirus. By integrating conserved CTL, HTL, and B-cell epitopes derived from immunodominant viral glycoproteins, a 70-amino-acid chimeric construct was successfully engineered:

NKKGEQVGMSRPGLKPDLTGTSKSYVCQHILM
KLMPNITLLPSTFMYGPPISLERLDVGTNLHHH
HHH

Comprehensive in silico analyses confirmed that the construct exhibits favorable antigenicity, non-allergenicity, non-toxicity, and structural stability. Physicochemical profiling indicated suitable molecular weight and solubility properties for recombinant expression, while tertiary structure modeling suggested acceptable conformational reliability for a short peptide immunogen. The inclusion of a C-terminal 6×His tag further enhances its experimental feasibility by facilitating purification and downstream characterization.

Importantly, the designed vaccine integrates epitopes capable of eliciting both humoral and cellular immune responses, targeting conserved regions of measles viral proteins associated with protective immunity. This rational design approach aligns with contemporary reverse vaccinology principles and offers potential advantages over traditional live-attenuated platforms, including improved safety and production flexibility.

Nevertheless, computational predictions require rigorous experimental validation. Future studies should include molecular docking with immune receptors, molecular dynamics simulations, codon optimization validation, recombinant expression, and immunogenicity assessment in suitable animal models. Additionally, formulation with appropriate adjuvants or nanoparticle-based delivery systems may enhance immunostimulatory efficacy.

In conclusion, the proposed multi-epitope peptide construct represents a promising next-generation vaccine candidate against measles. The findings provide a strong computational foundation for further translational and experimental investigations aimed at developing safe, stable, and scalable vaccine alternatives.

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