Análisis *in silico* de un candidato a vacuna multi-epítopo contra viruela del mono usando vaculonogía reversa

In silico analysis of a multi-epitope vaccine candidate against monkey pox using reverse vaccinology

Christian Fernando Montenegro Oyola[1,2]; Byron Alexander Noguera Rosero[1,2]; Juan Pablo García-López[1,2,3,4*]

1. Departamento de Biología, Universidad de Nariño, Pasto, Colombia.
2. Semillero de Investigación Biológica Aplicada a la Biodiversidad y a la Biotecnología en la Región (SIBIO), University of Nariño, Pasto, Colombia
3. Laboratorio de Biotecnología Microbiana, Universidad de Nariño, Pasto, Colombia.
4. Universidad Mariana, Pasto, Colombia.

Recibido: Agosto 20 de 2022
Aceptado: Noviembre 15 de 2022
*Correspondencia del autor: Juan Pablo García-López
E-mail: alcides. jpgarcia222@umariana.edu.co

https://doi.org/10.47499/revistaaccb.v1i34.265

Resumen

Introducción. La viruela del mono es una infección zoonótica con una tasa de transmisión global aumentada durante 2022. Actualmente, la enfermedad no tiene tratamientos específicos disponibles; por lo tanto, se puede lograr un enfoque preventivo a través de la inmunización. **Objetivo**. Diseño *in sílico* de una vacuna aplicando técnicas computacionales avanzadas utilizando una construcción de múltiples epítopos del *M. virus*. **Materiales y métodos**. Los antígenos se seleccionaron en base a informes sobre proteínas que provocan la activación de linfocitos T y B citotóxicos. Los ensayos inmunoinformáticos fueron antigenicidad, alergenicidad, toxicidad, afinidad de unión al complejo mayor de histocompatibilidad (CMH) y estimulación de IFN-γ. **Resultados y discusión**. Ocho epítopos de las proteínas M1R, ADN polimerasa, B6R y A35R de *M. virus* mostraron una respuesta significativa para las células inmunitarias. Se eligieron once epítopos con antigenicidad >0,3, no alergénicos y no tóxicos, de los cuales 4 presentaron alta afinidad por los linfocitos T, 4 generaron alta activación de linfocitos B y 3 se asociaron con resultados de activación de IFN-γ. La construcción *in sílico* del candidato vacunal de 509 aminoácidos con alta similitud topológica registró principalmente carga negativa, además de ser soluble con índice alifático >80%, estable y particular con activación CMH y alta afinidad molecular con TLR-3, y además presentó multiantigenicidad, similar a las vacunas generadas por esta metodología con *M. tuberculosis* e Influenza. La simulación de inyección de una dosis de la construcción molecular mostró la activación de las células plasmáticas auxiliares T durante aproximadamente 15 a 25 días y una alta expresión de IFN-γ e IL-2 durante ocho días. **Conclusión**. Estos resultados indican un excelente proceso de inmunización que podría potenciarse con dosis múltiples.

Palabras clave: Virus de la viruela del mono, vacuna, multiepítopo, simulación.

issn impreso 0120-4173, issn en línea 2500-7459

Abstract

Introduction. Monkey pox is a zoonotic infection with an increased global transmission rate during 2022, denoted epidemiological trouble in public health. Currently, the disease has no specific treatments available; thus, a preventive approach can be achieved through immunization. **Objective**. was to design in silico a vaccine applying advanced computational techniques using a multi-epitope construct of the *Monkeypox virus*. **Materials and methods**. Antigens were selected based on reports about proteins that cause the activation of cytotoxic T and B lymphocytes. The immunoinformatics assays were antigenicity, allergenicity, toxicity, MHC binding affinity, and IFN-γ stimulation. **Results and discussion**. Eight epitopes of the M1R, DNA polymerase, B6R, and A35R proteins of the *M. virus* showed a significant response for immune cells. Eleven epitopes with antigenicity >0.3, non-allergenic and non-toxic were chosen, of which 4 presented high affinity to T lymphocytes, 4 generated high activation of B lymphocytes, and 3 were associated with IFN-γ activation results. The *in silico* construction of the 509-amino acid vaccine candidate with high topological similarity registered mainly a negative charge, in addition to being soluble with an aliphatic index >80%, stable and particular with MHC activation and high molecular affinity with TLR-3, and also presented multi-antigenicity, similar to vaccines generated by this methodology with *M. tuberculosis* and Influenza. One-dose injection simulation of the molecular construct showed activation of T helper plasma cells for about 15 to 25 days and high expression of IFN-γ and IL-2 for eight days. **Conclusion**. These results indicate an excellent immunization process that could be potentiated with multi-dosing.

Keywords: Monkeypox virus, vaccine, multi-epitope, simulation.

Introduction.

Monkey pox is a zoonotic pathology that has increased its occurrence to 6.300 cases globally during July 2022 (1). This infection has been reported mainly in industrialized countries but is growing in developing countries such as the LATAM consortium. For example, until July 19, 6 cases were registered in Colombia, below Chile and Mexico, 20 and 48, respectively (Fig. 1A). This pathology records a lethality rate of 10% of infected people, with a greater affectation in children under ten years of age (2). The increase in infections is a possible consequence of immunocompromised people and past events such as COVID-19, in addition to the demographic displacement between countries (3,4). The aetiological agent, *Monkeypox virus* (5), is transmitted with a high infectivity rate, mainly to humans through wild animals and human-to-human contact (6,7). *M. virus* are large, enveloped, and brick-shaped (Fig. 1B), with some membrane glycoprotein used in experiments of molecular detection such as B6R (8). The pathological combat is focused on palliative care but without any specific treatments available; thus, the prevention of infection is a proper way of intervention, which can be achieved through community immunization (9).

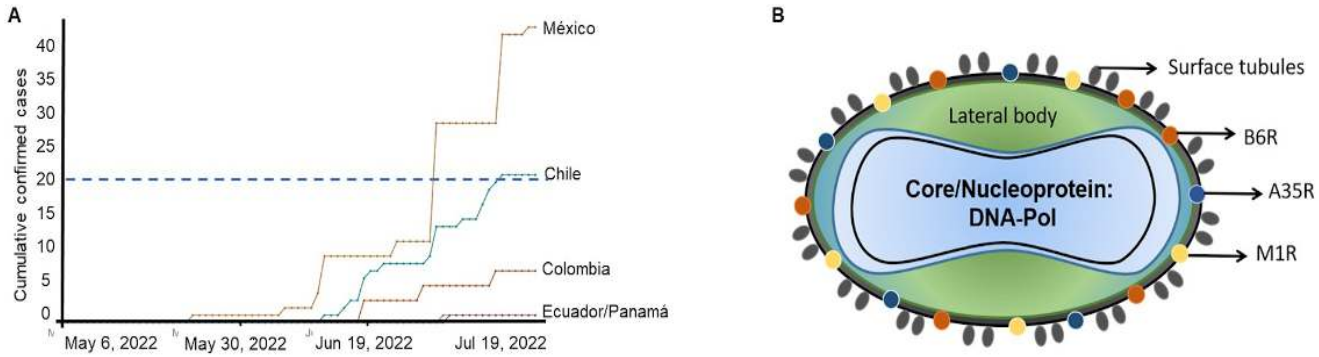

Figure 1. **Monkey pox statistics and biological characteristics.** A. Cumulated case confirmed of monkey pox in 5 LATAM countries. The blue dot line shows 20 cases to declare a health emergency country. B. Viral structure with proteins considered in the study.

First and second-generation vaccines are made with pathogenic antigens (10). These vaccines modulate the host defense system through activation and training of inflammatory mediators as INFγ, cellular level of innate immunity as macrophage, and acquired immunity as B and T lymphocytes (11,12). The interaction at the molecular level between the antigenic epitope and components of the immune system can be studied with in silico experiments (13). The evaluation of vaccines' efficacy with bioinformatics tools involves the evaluation of cell activation, humoral activation, antigenicity, allergenicity, toxicity, and structural and physicochemical characteristics (14). This approach has been studied to design a polyvalent vaccine built with antigenic epitopes against bacterial as *M. tuberculosis* (14), *Streptococcus pneumoniae* (15); a similar approach has been applied against some respiratory viruses such as SARS CoV 2 (16), and Syncytial virus (17). Some *in silico* design studies of vaccines to combat *M. virus* have been generated (18). However, reverse vaccinology studies result in many possible construct combinations from the same sequence database. In this context, the simulation experiments have been incorporated in immunoinformatics, to testing new reaches that some authors do not consider with other virus such as Corona B.1.617 Lineage (19); Although this route is usually common against *M. virus* (18, 19), the number of doses and the biological nature allows to generate diverse results.

Considering the COVID-19 pandemic actually and *M. virus* as a high-risk pathogen with global reach, the research efforts should be intensified to design biologicals used for pathological prevention, e. g. multi-epitope vaccines with a suitable activation level of acquired immunity cells, safety, stability, and few allergic and autoimmune events (20,21). Our research goal was to evaluate the *in silico* interaction of antigenic epitopes of *M. virus* with B and T lymphocytes and INFγ, in the characterization of a polyvalent vaccine. This study represents a novel and ideal method that allows evaluating alternative steps and saving laboratory work on issues of public health importance.

Methodology.

In this study, we use sequential pipeline bioinformatics, shown in figure 2. After extraction of the name of *M. virus* proteins, three ways were tested to probe the activation of T and B cells and the production of INFγ. The filter to selected epitopes is represented in the brown box with antigenicity, allergenicity, and toxicity test, with the main of joining the sequences to similar pipelines in other publications (18, 19). After obtaining the 3D Structure of the biological construct, antigenicity was also evaluated. Finally, the simulation experiments were included to check the T cell and antibody secretion with the doses.

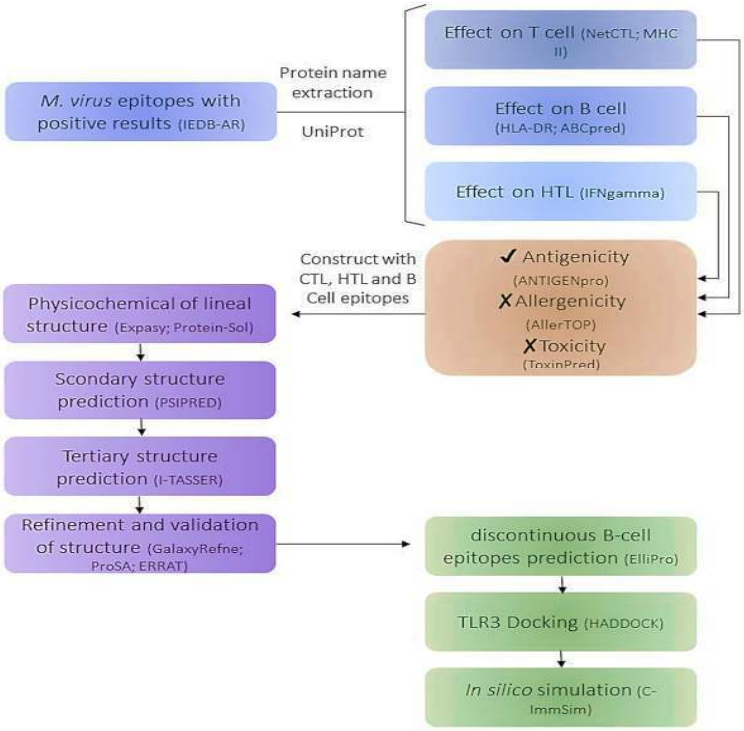

Figure 2. **Pipeline bioinformatic**. The color in the box represents congruent steps according to the sequences used. The arrows show the order in the sequential steps. The ✓ and ✗ symbols show the positive and negative expected results, respectively.

Sequences of consecution and interaction with CTL, B cells and INFγ.

The Immune Epitope Database and Analysis Resource (IEDB; http://www.iedb.org/) was used to select the Monkeypox virus antigenic epitopes. The amino acid sequences of proteins were obtained from UniProt (https://www.uniprot.org/): B6R (Q8V4S2), A35R (Q8V4U4), M1R (Q80KX3), and DNA polymerase (Q3I8V7). First, we analyzed these amino acid sequences to determine the activation of T and B cells and INFγ. Then, cytotoxic T lymphocyte activation level, TAP transport efficiency, C-terminal proteasomal cleavage, and epitope identification were executed using the NetCTL server (https://www.cbs.dtu.dk/services/NetCTL/) with thresholds 0.05, 0.15 and 0.75, respectively. Finally, the activation of helper T lymphocytes (HTL) was analyzed using the IEDB MHC II server (http://tools.iedb.org/mhcii/). The locus chosen for analysis was Human/HLA-DR, with a selection from a leukocyte antigen reference set of 7 alleles to predict epitopes with a length of 15 amino acids. HTL epitopes were chosen by activation of IFN-γ using the IFNgamma (15-mer) epitope server (http://crdd.osdd.net/raghava/ifnep itope/scan.php). Additionally, the activation of linear B lymphocytes was evaluated using ABCpred servers (http://www.imtech.res.in/raghava/abcpred/). For epitope identification, a window length of 16 mer was chosen, with a threshold value of 0.51. Epitopes with a score higher than 0.9 were selected. Second, the antigenicity was evaluated through the ANTIGENpro server (http://scratch.Proteomics.ics.uci.edu/). At the same time, the immunogenicity, conservation, and allergenicity were executed with the AllerTOP v.2.0 server (http:// www.ddg-pharmfac.net/AllerTOP), and toxicity using the ToxinPred server (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php). The peptides that activated cells and humoral mediators with good antigenicity, no toxicity, and no allergenicity were selected. The candidate vaccine was designed following Dorosti et al. (14); a trimer sequence and a Universal T helper activating sequence were included in addition to the adjuvant sequence (50s ribosomal protein L7/L12 NCBI ID:P9WHE3), joined by universal linkers.

Evaluation of physicochemical properties and prediction of 2D and 3D structures.

The physicochemical properties such as the theoretical isoelectric point (pI), amino acid composition, half-life in vitro and in vivo, instability and aliphatic number, molecular weight (MW), and grand mean hydropathicity (GRAVY) of the vaccine constructs, were evaluated with the Expasy Protparam server (https://web.expasy.org/protparam/). The solubility of the multi-epitope vaccine was predicted using the Protein-Sol server (http://protein-sol.manchester.ac.uk). The scaled solubility value greater than 0.45 will have a higher solubility than the average solubility. Secondary structure prediction was generated using the online tool PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/), which also predicts transmembrane topology, domain, fold recognition, and the transmembrane helix. The prediction model of the tertiary Structure of the multi-epitope vaccine was performed using the I-TASSER (Iterative Treading ASSEmbly Refinement) homology modeling tool server (https://zhanglab.ccmb.med. Umich.Edu/I-TASER/). A template modeling (TM) value > 0.5 shows a topology-accurate model, and a TM value < 0.17 indicates random similarity. Refinement is given by GalaxyRefne web server (http://galaxy.seoklab.org/cgi-binsubmit.cgi?type=REFINE), refining the 3D model obtained for the multi-epitope vaccine peptide. The validation of tertiary structures is a stage of identifying possible errors in the predicted 3D models. The ProSA web server (https://prosa.services.came.sbg.ac.at/prosa.php), was initially used for the validation of the 3D Structure of the protein. To investigate the associated non-bonded atom-atom interactions with the ERRAT web server (http://services.mbi.ucla.edu/ERRAT/) was also used to predict structures from high-resolution crystallography.

Antigenicity of vaccine construct, docking molecular and immune response simulation

For the prediction of discontinuous B-cell epitopes, we used the ElliPro online server (http://tools.iedb.org/ellipro/). ElliPro provides a score for each output epitope called the average PI value over each epitope. The ellipsoid with a PI value of 0.9 contains (90%) embedded protein residues, while the (10%) residues are outer ellipsoids. We executed docking molecular between the vaccine candidate and the immune receptor TLR3 (PDB ID: 1ZIW) using HADDOCK server (https://haddock.science.uu.nl/). In silico simulation of the immune response profile of the vaccine constrict was executed with method C-ImmSim server (http://150.146.2.1/C-IMMSIM/index.php), using one dose.

Results.

Epitope selection

Local government health systems have focused on preventing infection through self-care measures that accompany those adopted during the COVID-19 pandemic, for example, using face masks and avoiding tactile

contact with people who present lacerations or itching characteristics of monkeypox (22). The viral Structure allows noting potential antigens, e. g. in vitro experiments show four proteins of interest with activation results of components of the immune response, reported in the UniProt database. Among these proteins are the intracellular DNA-pol (Uniprot Q318V7), extracellular B6R (Uniprot Q8V4S2), A35R (Uniprot Q8V4U4), and M1R (Uniprot Q80KX3). These proteins were studied using reverse vaccinology computational techniques to design a polypeptide that works as an immunizer. The identification of epitopes antigenic was executed by activation of lymphocytes and inflammatory mediators, at the same time, the study of their degree of toxicity and antigenicity. The results show 15 peptides with a binding capacity to MHC-I, with a Gibbs energy greater than 0.5, indicating activation of cytotoxic T lymphocytes (CTL); this limit was defined according to results reported by this technical literature for the *Mycobacterium tuberculosis* (13). In this group, 13 peptides presented a level of antigenicity greater than the 0.15 limit, which outlines them as molecules that would allow training of the immune response through its activation.

Sequentially, seven peptides showed in silico toxicity, which was discarded in the selection of candidates (Fig. 3A). 4 peptides, one from each protein tested, were selected considering the highest levels of MHC-I activation and antigenicity. Eight peptides of each protein were studied to test the activation of B lymphocytes. Figure 3B indicates all activated B cells with a score greater than 0.5. Although in this group, all the peptides presented antigenicity greater than 0.2; 3 molecules were discarded for being presumably toxic to the organism. As in the previous experiment, four peptides were selected, 1 of each tested protein having as criteria the highest B-cell activation and antigenicity. To the INFγ mediator, four peptides were tested, which showed antigenicity and activation of HTL cells capable of synthesizing the INFγ mediator. However, the epitope of the M1R protein was toxic, so it was discarded (Fig. 3C). These results were incorporated into the designed polypeptide shown in Figure 3D. The strategy presented by Bibi et al. (14) was followed, and the trimer and universal T helper sequences were added to increase the possibility of activation of this cell subpopulation and consequent modulation of the immune response. The linear sequence was organized with the adjuvant peptides, joined by the amino acid linker EAAAK, which interacts with the polypeptide to simulate the vaccine dose. Initially, the B cell and HTL activating epitopes were linked via the linker amino acids GPGPG, followed by the CTL activating epitopes linked via the linker amino acids AAY. In the end, a polypeptide of 509 residues was obtained.

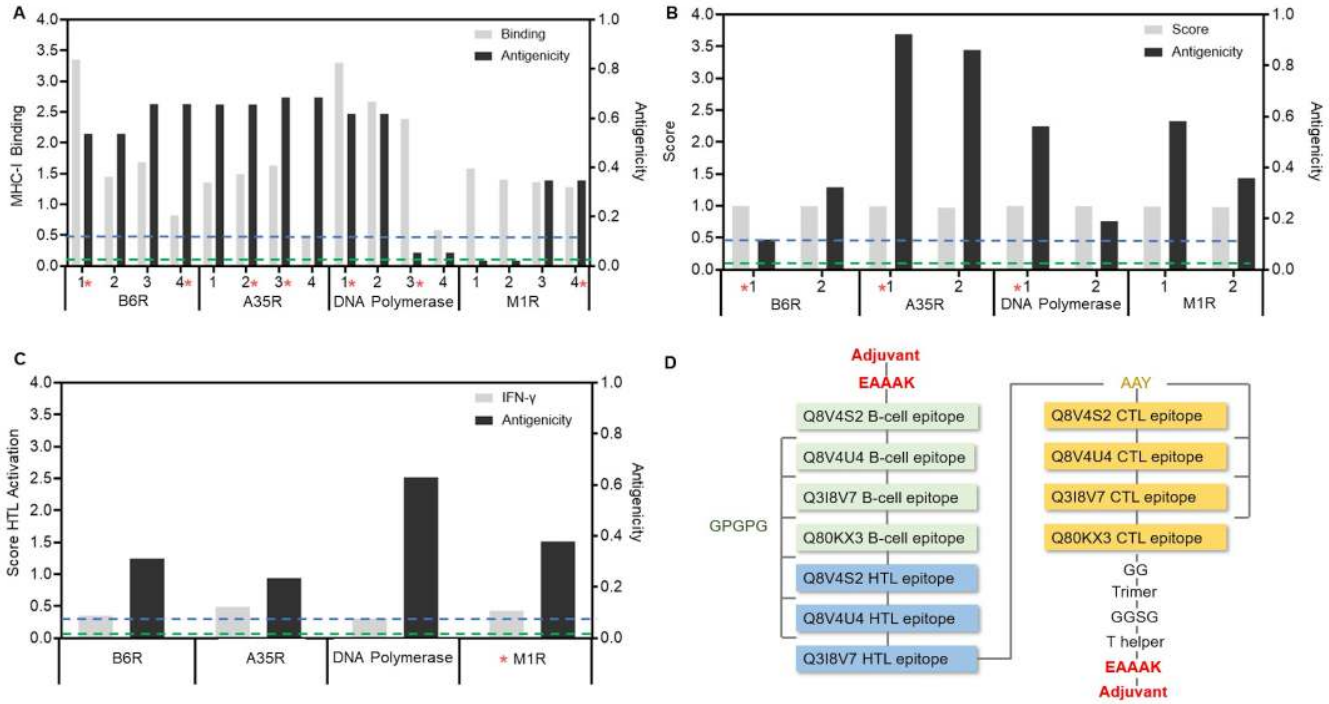

Figure 3. **Selection of peptides to vaccine construct**. The score of the antigenicity and MHC-I binding (A), B cells activation (B), and HTL activation (C). The allergenic peptides are shown with a red asterisk. The threshold of the score for each category (blue dot line) and antigenicity (green dot line) are shown (D). Organization of sequences for vaccine construct. The linkers between sequences are shown

Characterization of vaccine construct.

Physicochemical and structural tests studied the characterization of the polypeptide. The three-dimensional robustness was also evaluated, and the ability to react as a protein was studied. Initially, the lines structure of the designed candidate showed a solubility index greater than 0.6 according to the criteria on the page (Fig. 4A), indicating hydrophilicity and the ability of the polypeptide to be diluted in aqueous environments. The electrostatic composition indicates mostly negatively charged amino acids, with a score between -0.05 and -0.25. Short regions indicated a positive charge up to 0.15. At the same time, most regions have plegation capacity with a window between 0 and 0.715 (Fig. 4B). The three-dimensional Structure was built by homology following the I-TASSER server algorithm. Four models were found, of which two were selected by modeling scores of -1.56 and -2.98, respectively. In Figure 4C, the presence of beta sheets and alpha helices connected by regions with no folding is mostly shown. This Structure was confirmed by the iterative threads´ criterion, which uses the I-TASSER algorithm. A confirmation index of 0.9396 was recorded, where conserved regions can be seen (Fig. 4D) of the first Structure, revealing statistical robustness in the protein modeling. This 3D Structure was checked against databases to determine the closeness to already crystallized proteins. As indicated in figure 4E, the generated polypeptide has an affinity with reported structures on the X-ray basis, with a Z-score of -4.8. The energy analysis according to the position of the peptide shows an oscillation between 0.9 and -1.5 (Fig. 4F). This allows us to predict that the behavior of the protein in aqueous environments would generate electrostatic attraction with cells and molecules with cationic and anionic charge.

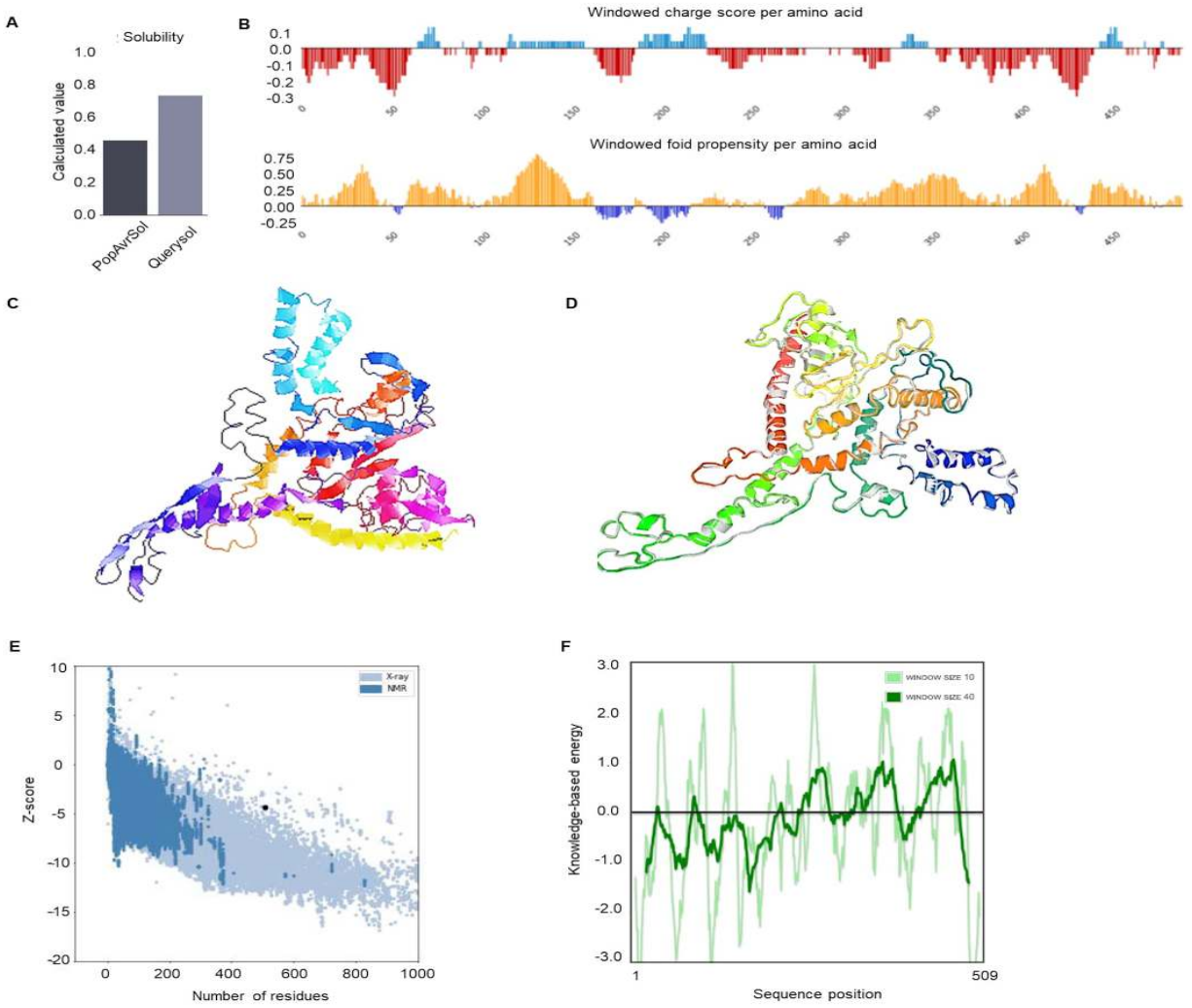

Figure 4. Physicochemical and structural characterization of vaccine construct. A. Calculates solubility value using two algorithms. B. Charge score (upper) and foil propensity (lower) per amino acid position. 3D Structure of the first model (C) and refined model (D). E. Z-score for comparison of vaccine constructs with X-ray and NMR database. F. Windowed of energy per amino acid.

Simulation of immune response with vaccine construct.

The activation of TCR receptors that are expressed in cells of the defense system would indicate modulation of the immune response. To test the molecular interaction of the designed polypeptide, the molecular interaction of the protein was observed by the molecular docking method with the TCR receptor. The results indicate a Gibbs energy index of -6102.60, which is represented in Figure 5A, where covalent bonding between amino acids of the polypeptide with the TCR receptor can be seen. The activation of the immune response was studied by means of a dosage simulation to demonstrate the immunizing effect. A single dose of the polypeptide was tested, and the behavior of T cells, mainly immunoglobulins, was observed. The results indicate that T helper or Th1 cells increase four days after injection, reaching 34.000 cells/mm3. This number of cells remains up to 32 days post-injection (Fig. 5B). Figure 5C shows the synthesis of immunoglobulins IgM, IgG1, and IgG2 at levels 500.000, 150.000, and 50.000, respectively.

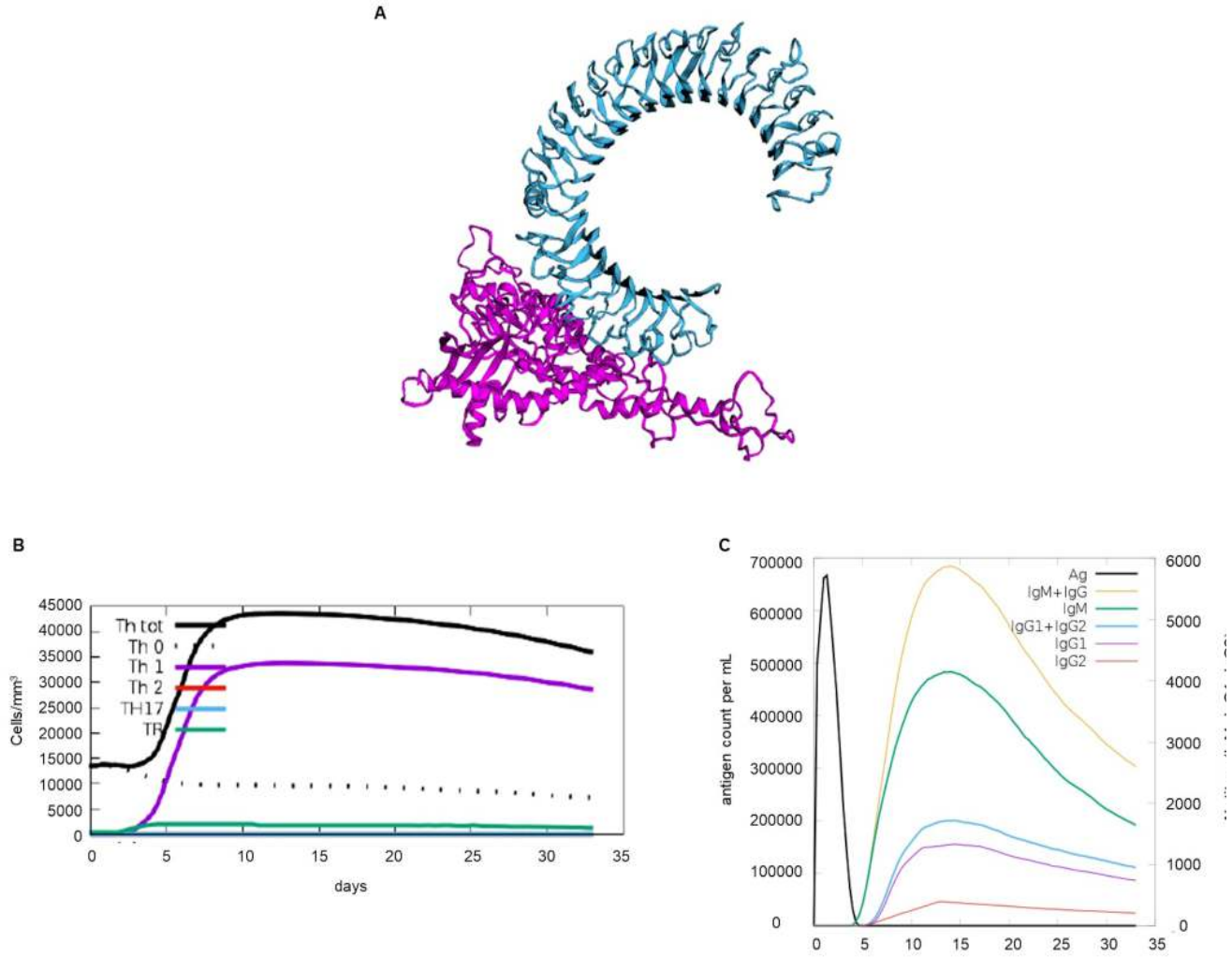

Figure 5. Molecular interaction with TCR receptor and simulation of immune response with vaccine construct. A. Docking molecular between TCR (blue structure) and vaccine construct (purple structure). B. T helper by mm^3 after dose application for 35 days. C. Antigen and Ig count per Ml after dose application for 35 days.

The CTL binding affinity of peptides ranges from 0.3039 to 3.3551 (Table 1, Supplement 2), HTL binding affinity ranges from approximately 1.5 to 4.2 (Table 2, Supplement 2), and for gamma interferon, all peptides have a score of 0.9 (Table 3, Supplementary 2).

Together, these results contrast with the literature because the number of epitopes is lower than those reported by Bibi et al. (13) for tuberculosis. However, they show stimulation of the immune system generating an immunization process without considering the size of the construct of the vaccine at the *in silico* modeling level against *M. virus*. According to the results revealed by the simulation of the immune response, it can be observed that there is a behavior similar to that reported with pathogens of interest in public health, such as *M. tuberculosis* (14), revealing that immunization has a positive effect on the host and can thus minimize and combat the risk of specific pathogens, thus showing effectiveness and optimum balance in the tests carried out for modeling the vaccine against the *M. virus*

Discussion.

The National Institute of Allergy and Infectious Diseases classifies the *Monkeypox virus* as a re-emerging pathogen from its first recorded in 1958, with prominent transmission and considerable infection rate in humans (23). The actual immunization plan to prevent Monkeypox considers the vaccine type as JYNNEOS™, also known as Imvamune or Imvanex, supported by significant studies of NIAID (24). However, new biologicals must be applied to stimulate both humoral and cellular immune responses. This is in line with the strategy during the COVID-19 pandemic with the distribution of vaccines from various supplier companies like Comirnaty®, Moderna, Janssen, among others. The course of transmission of SARS CoV 2, the etiological virus of COVID-19, occurs with mutations in its genotype resulting in lineages and variants that can be convinced with different effectiveness by a range of vaccines (25). The immunoinformatics approach is beneficial in developing multi-epitope vaccines (26). The historical success of reverse vaccinology has shown great public interest in the global transmission of emerging and re-emerging pathogens during the COVID-19 pandemic.

The publications that are increasing with the methods of in silico design of vaccines against Monkey pox anticipates a scenario to combat the appearance of new variants of the *M. virus*.

The bioinformatics pipeline reported to *in silico* design vaccine directed to pathogens combat take different ways to the same main: immunization. These methods attach three points: 1) the source of sequences, 2) the assembly of sequences analyzed, and 3) the deep of the immunoinformatics algorithms approach. The sequences are usually extracted from previous literature or comprehensive databases like GenBank, RCBS PDB, or IEDB-AR. The IEDB-AR from NIAID is a database—analysis resource that presents a catalog of experimental antibody and T cell epitopes studied in diverse species during an immune response and tools to assist in the analysis of epitopes (27). The easy access to information from the IEDB-AR database yields promising results for target pathogens such as *Trypanosoma cruzi*, *Ebola virus*, and Dengue virus, among others (28-30). In this study, IEDB-AR was used to search the name of proteins that have epitopes reported with significant positive in vivo and *in vitro* results: T-cell quantification in *Macaca mulatta* as host of *Monkeypox virus* (31); *In vitro*, IFN gamma release detected with ELISPOT and ICS assays (32); and MHC quantification thorough ELISA test (33). After this report, the lineal Structure (all amino acids) of B6R, A35R, M1R, and DNA-Pol proteins were evaluated for epitope selection; this allows the evaluation of new sequences not considered in the database.

On the other hand, the assembly of selected sequences can lead to many combinations that support new configurations of the same vaccine. Finally, some labs assess different immunoinformatics algorithms to demonstrate immunological reactivity by alignment with previous reports and docking molecular (19). However, few studies extend the analysis to the simulation of vaccine doses (18,34).

We applied in identifying vaccine candidates using the strategy report by Shantier et al. (34) using reverse vaccinology to target *M. tuberculosis* infection with 35 bibliographic citations, who add the immune simulation approach in the *in silico* design of biological. The refined Structure of the construct shows consistency with the homology-adjusted models provided by I-TASSER. Our molecular docking results show an index of Gibbs energy 10%, 20%, and 50% more than what was reported by Abdi et al. (18) against *M. virus*; Omoniyi et al. (35) against the Crimean-Congo virus; and Nelluri et al., (36) against *Bunyumwera virus*, respectively. The level could be attributed to the inclusion of CTL activating epitope sequences (37), and the universal adjuvant

has been reported to stimulate TLR-4 and B cells. To test the protection level of cell memory immunological with the multi-epitope construct, we show accurate molecular dynamics through immune stimulation in Fig. 5. consistent with Th1 T cells dynamic after vaccine doses, our result showed as subpopulation Th1 cells relevant by their cytokines secretion as IFN-γ to increase the humoral protection level (38) against *M. virus*. Other inflammatory mediators, as antibodies represented in Fig. 4, result in a similar amount to the study of Abdi et al. (18); however, it is recommended in future studies that *in silico* simulations consider multi-dosing (35).

Conclusion.

The immuno-informatic approach in this study allows new knowledge about antigenic epitopes and vaccine candidates against *M. virus*, which can be synthesized in subsequent stages of *in vitro* preclinical research. A high antigenicity level and good stability in immune-scene simulations characterize our vaccine construct. Furthermore, evaluating isolated epitopes and multi-epitope vaccine constructs denotes increased immunogenicity in the polypeptide potential to combat Monkey pox disease.

Declarations.

Acknowledgements.

The authors thank the support in the design of research to Pablo Fernández and Fedra Ortíz.

Author Contributions.

Conceptualization and Methodology, all authors; Data Curation and Validation: JPG; Formal Analysis, all authors; Writing-Original Draft Preparation, JPG; Writing-Review and Approval, all authors. All authors have read and agreed to the published version of the manuscript.

Funding.

The authors gratefully acknowledge funding by the Departamento de Biología de la Universidad de Nariño and Semillero de Investigación Biológica Aplicada a la Biodiversidad y a la Biotecnología en la Región (SIBIO).

Competing interests.

The authors declare that they have no conflict of interest with funders or personal relationship that could have had a bearing on the work presented in this paper.

Ethical Approval.

All procedures followed the ethical standards of Helsinki Declaration of 1964 and its later amendments.
Consent to Participate. Not applicable.

Referencias

1. Peter OJ, Kumar S, Kumari N, Oguntolu FA, Oshinubi K, Musa R. Transmission dynamics of Monkeypox virus: a mathematical modelling approach. Model Earth Syst Environ. 2021; 1:1–12.
2. Nguyen PY, Ajisegiri WS, Costantino V, Chughtai AA, MacIntyre CR. Reemergence of Human Monkeypox and Declining Population Immunity in the Context of Urbanization, Nigeria, 2017–2020. Emerg Infect Dis. 2021; 27(4):1007.
3. Durski KN, McCollum AM, Nakazawa Y, Petersen BW, Reynolds MG, Briand S, et al. Emergence of Monkeypox — West and Central Africa, 1970–2017. Morb Mortal Wkly Rep. 2018, 67(10):306.
4. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al. Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. Lancet Infect Dis. 2019; 19(8):872–9.
5. Vaughan A, Aarons E, Astbury J, Brooks T, Chand M, Flegg P, et al. Human-to-Human Transmission of Monkeypox Virus, United Kingdom, October 2018. Emerg Infect Dis. 2020; 26(4):782.
6. Alakunle E, Moens U, Nchinda G, Okeke MI. Monkeypox Virus in Nigeria: Infection Biology, Epidemiology, and Evolution. Viruses. 2020, 12(11):1257.
7. Saijo M, Ami Y, Suzaki Y, Nagata N, Iwata N, Hasegawa H, et al. Virulence and pathophysiology of the Congo Basin and West African strains of monkeypox virus in non-human primates. J Gen Virol. 2009, 90(9):2266–71.
8. Li Y, Olson VA, Laue T, Laker MT, Damon IK. Detection of monkeypox virus with real-time PCR assays. J Clin Virol. 2006; 36(3):194-203.
9. Moxon ER, Siegrist CA. The next decade of vaccines: societal and scientific challenges. Lancet. 2011; 378(9788):348–59.
10. Guevara JR., Buelvas N, Suárez R, Peña L, Chirinos R., Pérez I, Urdaneta H. Esfuerzos para el desarrollo de vacunas y adyuvantes. Acta Bioclínica. 2018; 8(15), .
11. Céspedes PF, Cruz P, Co N. Modulación de la respuesta inmune durante la infección por virus distemper canino: implicancias terapéuticas y en el desarrollo de vacunas. Arch Med Vet. 2010; 42:15–28.
12. Oli AN, Obialor WO, Ifeanyichukwu MO, Odimegwu DC, Okoyeh JN, Emechebe GO, et al. Immunoinformatics and Vaccine Development: An Overview. ImmunoTargets Ther. 2020; 9:13.
13. González-Romo F, Picazo JJ. El desarrollo de nuevas vacunas. Enferm Infecc Microbiol Clin. 2015; 33(8):557–68.
14. Bibi S, Ullah I, Zhu B, Adnan M, Liaqat R, Kong WB, et al. In silico analysis of epitope-based vaccine candidate against tuberculosis using reverse vaccinology. Sci Reports 2021; 11(1):1–16.
15. Dorosti H, Eslami M, Nezafat N, Fadaei F, Ghasemi Y. Designing self-assembled peptide nanovaccine against Streptococcus pneumoniae: An in silico strategy. Mol Cell Probes. 2019; 48:101446.
16. Jahangirian E, Jamal GA, Nouroozi M, Mohammadpour A. A reverse vaccinology and immunoinformatics approach for designing a multiepitope vaccine against SARS-CoV-2. Immunogenetics. 2021; 73(6):459-477.
17. Tahir Ul Qamar M, Shokat Z, Muneer I, Ashfaq UA, Javed H, Anwar F, Bari A, Zahid B, Saari N. Multiepitope-Based Subunit Vaccine Design and Evaluation against Respiratory Syn-

cytial Virus Using Reverse Vaccinology Approach. Vaccines. 2020; 8(2):288.
18. Abdi SAH, Ali A, Sayed SF, Abutahir, Ali A, Alam P. Multi-Epitope-Based Vaccine Candidate for Monkeypox: An In Silico Approach. Vaccines. 2022; 10(9):1564.
19. Seadawy MG, Zekri ARN, Saeed AA, San EJ, Ageez AM. Candidate Multi-Epitope Vaccine against Corona B.1.617 Lineage: In Silico Approach. Life. 2022; 12(11):1715.
20. Zhang L. Multi-epitope vaccines: a promising strategy against tumors and viral infections. Nat Publ Gr. 2017; 15:182–4.
21. Negahdaripour M, Nezafat N, Eslami M, Ghoshoon MB, Shoolian E, Najafipour S, et al. Structural vaccinology considerations for in silico designing of a multi-epitope vaccine. Infect Genet Evol. 2018; 58:96–109.
22. Gomez De la Rosa RJ, Rojas JP. Viruela del simio: ¿estamos frente a un riesgo de pandemia?. Historia de un viejo conocido. Interdiscip J Epidemiol Public Heal. 2022; 5(2).
23. Parker S, Buller RM. A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012. Future Virol. 2013; 8(2):129-157.
24. Payne AB, Ray LC, Cole MM, Canning M, Houck K, Shah HJ, et al. Reduced Risk for Mpox After Receipt of 1 or 2 Doses of JYNNEOS Vaccine Compared with Risk Among Unvaccinated Persons - 43 U.S. Jurisdictions, July 31-October 1, 2022. MMWR Morb Mortal Wkly Rep. 2022; 71(49):1560-1564.
25. Gregory DA, Trujillo M, Rushford C, Flury A, Kannoly S, San KM, et al. Genetic diversity and evolutionary convergence of cryptic SARS- CoV-2 lineages detected via wastewater sequencing. PLoS Pathog. 2022; 18(10):e1010636.
26. Martinelli D. In silico vaccine design: A tutorial in immunoinformatics. Healthcare Analytics. 2022; 2:100044.doi.org/10.1016/j.health.2022.100044.
27. Dhanda SK, Mahajan S, Paul S, Yan Z, Kim H, Jespersen MC, et al. IEDB-AR: immune epitope database-analysis resource in 2019. Nucleic Acids Res. 2019; 2;47(W1):W502-W506.
28. Trevisan RO, Santos MM, Desidério CS, Alves LG, de Jesus Sousa T, de Castro Oliveira L, et al. In Silico Identification of New Targets for Diagnosis, Vaccine, and Drug Candidates against Trypanosoma cruzi. Dis Markers. 2020; 2020:9130719. doi: 10.1155/2020/9130719.
29. Liu Y, Sun B, Pan J, Feng Y, Ye W, Xu J, et al. Construction and evaluation of DNA vaccine encoding Ebola virus glycoprotein fused with lysosome-associated membrane protein. Antiviral Res. 2021; 193:105141.
30. Abesamis LMI, Aliping EGA, Armada FKGH, Danao MF, Del Valle PDB, Regencia ZJG, et al. In Silico Comparative Analysis of Predicted B Cell Epitopes against Dengue Virus (Serotypes 1-4) Isolated from the Philippines. Vaccines. 2022; 10(8):1259.
31. Song H, Josleyn N, Janosko K, Skinner J, Reeves RK, Cohen M, et al. Monkeypox virus infection of rhesus macaques induces massive expansion of natural killer cells but suppresses natural killer cell functions. PLoS One. 2013; 8(10):e77804.
32. Heraud JM, Edghill-Smith Y, Ayala V, Kalisz I, Parrino J, Kalyanaraman VS, et al. Subunit recombinant vaccine protects against monkeypox. J Immunol. 2006; 177(4):2552-64.
33. Sidney J, Asabe S, Peters B, Purton KA, Chung J, Pencille TJ, Purcell R, Walker CM, Chisari FV, Sette A. Detailed characterization of the peptide binding specificity of five common Patr class I MHC molecules. Immunogenetics. 2006; 58(7):559-70.
34. Shantier, S.W., Mustafa, M.I., Abdelmoneim, A.H. et al. Novel multi epitope-based vaccine against monkeypox virus: vaccinomic approach. Sci Rep. 2022; 12:15983.
35. Omoniyi, A.A., Adebisi, S.S., Musa, S.A. et al. In silico design and analyses of a multi-epitope vaccine against Crimean-Congo hemorrhagic fever virus through reverse vaccinology and immunoinformatics approaches. Sci Rep. 2022; 12:8736.

36. Nelluri KDD, Ammulu MA, Durga ML, Sravani M, Kumar VP, Poda S. In silico multi-epitope Bunyumwera virus vaccine to target virus nucleocapsid N protein. J Genet Eng Biotechnol. 2022; 20(1):89. Doi: 10.1186/s43141-022-00355-y
37. Ahlers JD, Belyakov IM, Thomas EK, Berzofsky JA. High-affinity T helper epitope induces complementary helper and APC polarization, increased CTL, and protection against viral infection. J Clin Invest. 2001; 108(11):1677-85.
38. Gupta G, Shareef I, Tomar S, Kumar MSN, Pandey S, Sarda R, Singh R, Das BK, Sinha S. Th1/Th2/Th17 Cytokine Profile among Different Stages of COVID-19 Infection. Natl Acad Sci Lett. 2022; 45(4):363-369.

Supplementary

Supplementary 1: https://drive.google.com/file/d/17TCG-H2fMd2zsUsyY08PT1PFTDaji9Vf/view?usp=sharing

Supplementary 2: https://drive.google.com/file/d/1_Bmy8eLNtgvoP29wDbacC3FvFdYzN-Nxp/view?usp=sharing