MANUSCRIPT TITLE

VaccineDesigner: A Web-based Tool for Streamlined Epitope-based Vaccine Design

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ABSTRACT

Epitope-based vaccine design is a promising alternative to conventional methods, focusing on selected antigenic epitopes and molecular fragments that can interact with the immune system and elicit appropriate immune responses. Computational epitope-based vaccine design provides an efficient strategy for immunogenic and safe vaccines; however, the implementation of this approach requires the integration of heterogeneous tools with limited interoperability. VaccineDesigner addresses this issue by offering a user-friendly platform that integrates methods for predicting and evaluating B-cell, CTL, and HTL epitopes. The goal is to provide a Web-based interface for integrated analyses, empowering fast, cost-effective, and rationalized multi-epitope vaccine design in a streamlined process. VaccineDesigner is based on a modular architecture that seamlessly integrates B-cell, CTL, and HTL epitope prediction tools and scoring algorithms, along with multi-epitope vaccine sequence generation. VaccineDesigner is an open-source tool freely available under the academic license at: https://github.com/BiolApps/VaccineDesigner. The Web-based application is accessible at: http://bioinformatics.med.auth.gr/VaccineDesigner

INTRODUCTION

Developing efficacious vaccines against a range of pathogens is a primary research priority in the fields of immunology and public health. Vaccination, a cornerstone of preventive medicine,

has historically transformed global healthcare and reduced disease burdens by saving lives (1). Conventional vaccine development relies on live attenuated pathogens, inactivated agents, subunit formulations, or recombinant protein antigens, facing challenges such as pathogen cultivation and safety concerns (2).

Epitope-based vaccine design offers a promising alternative, focusing on the use of selected antigenic epitopes and molecular fragments that engage with the immune system. These epitopes include B-cell epitopes for humoral responses, cytotoxic T lymphocyte epitopes for cellular immunity, and helper T lymphocyte epitopes for immune regulation (3). B-cell epitopes consist of surface-accessible clusters of amino acids and activate immune responses through antibody production (4). T-cell epitopes consist of small peptide fragments and activate immune responses by presentation to antigen-presenting cells (APCs) through MHC class I or class II molecules. Cytotoxic T cells respond to MHC class I-restricted peptides, called CTL epitopes, whereas Helper T cells target MHC class II-restricted peptides, referred to as HTL epitopes (5). Vaccines combining these components have the unique ability to simultaneously activate both humoral and cellular immunity while avoiding the presence of elements that could provoke harmful reactions (6).

Bioinformatics is an indispensable ally in advancing our understanding of immunology and vaccine development by providing the means to predict and strategically harness epitopes. Over the past decade, a comprehensive set of tools and methods has been developed across various facets of the immune response, encompassing B-cell, CTL, and HTL epitope prediction (7).

Although considerable efforts have been made to harness Artificial Intelligence in the multi-epitope sequence design process, there is still a notable gap in the field of bioinformatics tools (8). Several tools and algorithms have been developed, each targeting specific aspects of epitope prediction, such as B-cell, CTL, or HTL epitopes (9,10). These tools are available through heterogeneous interfaces and most of them lack interoperability features. Consequently, researchers face the challenge of combining the functionalities of several standalone tools, each with different interface and input requirements, to design multi-epitope vaccine sequences. In this context, the need to orchestrate these tools through a unified and user-friendly interface would offer a highly desirable potential for innovation and advancement in epitope-based vaccine development.

This work presents VaccineDesigner, a Web-based tool that aims to bring flexible and rational epitope-based vaccine design applications to a broader audience. VaccineDesigner offers a streamlined and user-friendly experience, requiring researchers to provide nothing more than FASTA files containing their meticulously selected proteins. This simplified input process eliminates the need for intricate programming and data manipulation. Under the hood, VaccineDesigner processes protein sequence data and seamlessly integrates the predictive prowess of B-cell, CTL, and HTL epitope methodologies.

MATERIAL AND METHODS

The main components of the workflow involve epitope prediction for B-cell and T-cell Lymphocytes (Fig. 1), as crucial indicators of protein antigenicity (9). The application leverages Bepipred for B-cell epitope prediction (10), NetMHCpan for MHC class I and II (11), or Consensus IEDB tool for binding prediction (12). The predicted epitopes undergo subsequent filtering steps, assessing their antigenicity, toxicity, and allergenicity according to user-defined criteria, as shown below.

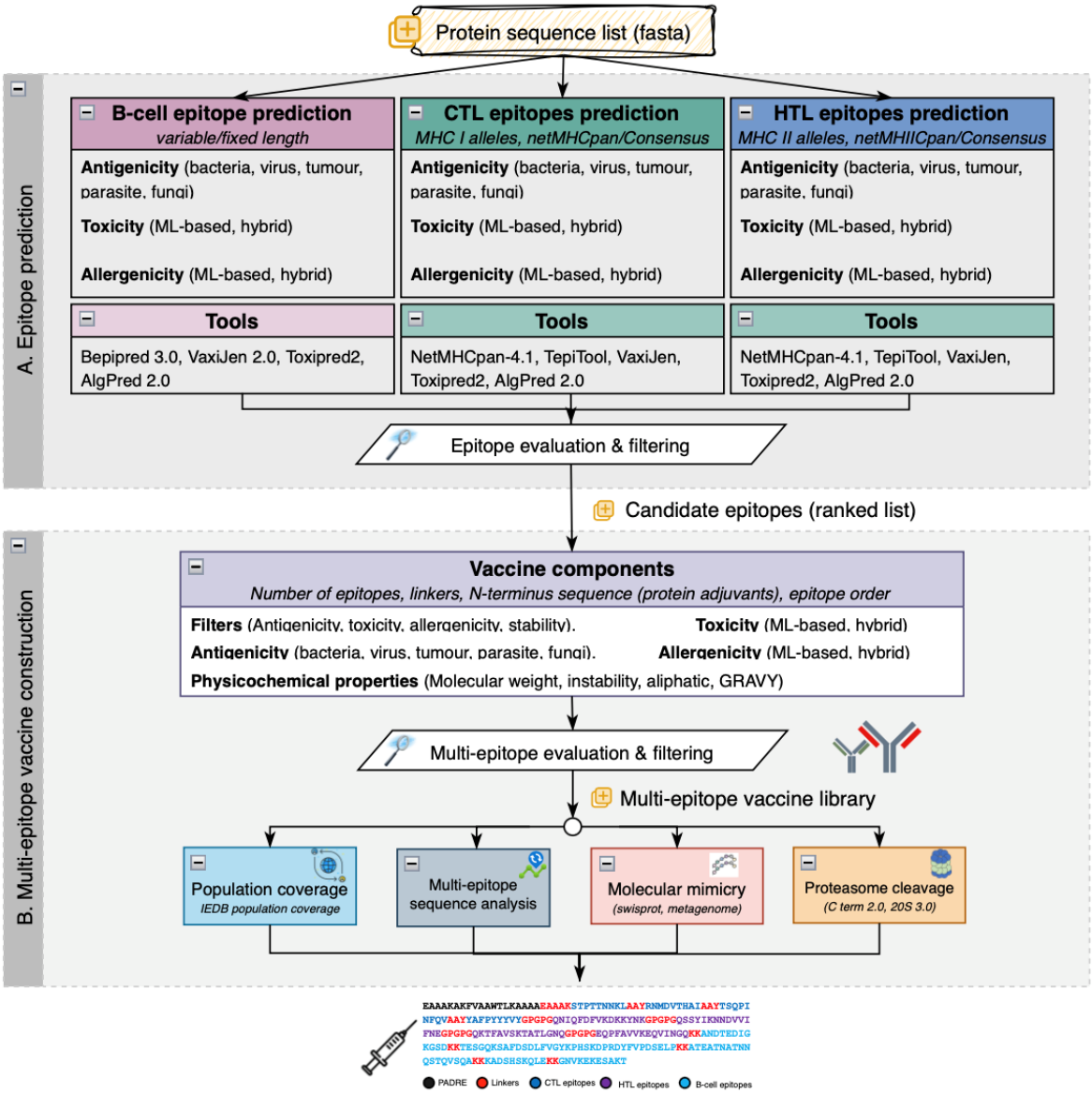

Figure 1

Epitope Prediction

VaccineDesigner starts by uploading a FASTA protein file with user-selected proteins (Fig. 1). Users are then prompted to select a protein of interest to proceed with epitope prediction. In the VaccineDesigner workflow, the epitope prediction module is initiated as an integral component of epitope-based vaccine design that harnesses well-established algorithms, such as BepiPred 3.0, NetMHCpan, and NetMHCIIpan, to serve various epitope prediction purposes.

B-cell epitope prediction. B-cell epitope prediction uses BepiPred 3.0. BepiPred systematically scans protein sequences and assesses key physicochemical properties and sequence patterns to identify regions that can induce robust antibody responses (10). VaccineDesigner uses the amino acid scores and generates B-cell epitopes, adhering to user-defined epitope length parameters. Two types of analysis can be deployed. The first type involves the formation of B-cell epitopes in high-scoring regions with no upper length limit but with low length, tuned by the *Minimum Epitope Length* parameter. The *Subthreshold Amino Acid Inclusion Count* parameter determines the maximum number of amino acids between predicted epitopes to be included in the output, allowing the merging of separate epitopes into a larger epitope region. To avoid low-scoring amino acids from being included in the results, VaccineDesigner uses the *Secondary Threshold* parameter that sets a minimum amino acid score to be included in the epitope prediction. The second type of analysis pertains to the generation of B-cell epitopes of user-defined lengths. Upon user input, a scanning window of the specified epitope length assesses the scores of all amino acids. Epitopes are defined as continuous regions of 9 or more amino acids, wherein each position possesses a score equal to or exceeding the threshold value. The B-cell epitopes are presented in ranked order based on the mean Bepipred score of the epitope region, arranged in decreasing order.

CTL epitope prediction. CTL epitope prediction in VaccineDesigner is implemented by NetMHCpan 4.1 (11) or the Consensus Method by IEDB Class I prediction (12). NetMHCpan is an algorithm renowned for its accuracy in identifying high-affinity regions for MHC class I alleles selected by the user. The output includes both strong and weak binders counts along with detailed information about the associated MHC alleles. On the other hand, the IEDB Consensus method stands as a reputable algorithm in forecasting CTL epitopes. This method seamlessly integrates various bioinformatics tools, harnessing their combined capabilities to elevate the accuracy of epitope prediction. The CTL epitopes are presented in descending order based on the number of alleles interacting with each respective epitope.

HTL epitope prediction. The HTL epitope prediction module relies on NetMHCIIpan 4.0, that employs the same machine learning framework as NetMHCpan 4.1. NetMHCIIpan identifies regions characterized by strong binding affinities for MHC class II molecules, customizable to specific user-defined MHC class II alleles (11). The results, akin to CTL epitopes, include strong and weak binders counts, along with details of the MHC alleles. Additionally, HTL epitope

prediction can be executed using the IEDB Consensus method for class II binding prediction, employing the same framework as the Class I module. HTL epitopes are similarly ranked based on the number of interacting alleles, mirroring the approach used for CTL epitopes.

Epitope Evaluation and Filtering

After epitope prediction, VaccineDesigner includes an optional step that performs epitope evaluation and filtering with VaxiJen (13), ToxinPred (14), and AlgPred (15). VaccineDesigner ensures the meticulous selection of epitopes with exceptional antigenicity and safety profiles, adhering to user-defined thresholds. With VaxiJen, a software tool known for its high prediction accuracy rates, VaccineDesigner assesses the antigenicity of each epitope, prioritizing highly immunogenic candidates. ToxinPred identifies potential toxins within epitope sequences, serving as a crucial safety measure. AlgPred predicts allergenicity, minimizing the risk of allergic reactions in vaccine recipients. This comprehensive evaluation guarantees the selection of epitopes based on user-defined criteria for antigenicity and safety, thereby improving the overall efficacy and safety of candidate multi-epitope constructs.

Multi-epitope Vaccine Sequences

VaccineDesigner includes a module that employs epitope predictions to craft multi-epitope vaccine sequences. Using the most prominent epitopes, VaccineDesigner builds larger constructs by merging these epitopes with appropriate linker sequences (default: GPGPG/HTL, AAY/CTL, KK/B-cell) (3,16–21). These multi-epitope constructs are known to mitigate junctional immunogenicity and efficient epitope separation and presentation to T-cell and B-cell receptors (3). The analysis is implemented in a two-step procedure:

Vaccine Sequences Construction. VaccineDesigner grants control over the development of multi-epitope vaccine sequences. Users are prompted to define the number of epitopes for B-cell, CTL, and HTL, while also specifying user-defined linker sequences (18) between the epitopes and the N-terminus sequence. A variety of different options of protein adjuvants are provided for the user to select and include in the multi-epitope vaccine sequences (22–25).

Additionally, the order of epitope combination can be defined, enabling precise customization, such as arranging B-cell, CTL, and HTL epitopes in a specific sequence, exemplified as B-C-H (B cell - CTL - HTL). VaccineDesigner enables the generation of candidate vaccine sequences, which form a versatile library constructed through epitope sequence combinations. Researchers have the flexibility to explore diverse epitope combinations, with the only constraint being a maximum limit of five epitopes from each category (B-cell, CTL, and HTL).

Vaccine Sequences Evaluation and Selection. In the final step, the constructed candidate vaccine sequence library undergoes rigorous evaluation. The algorithm assesses each sequence against predefined filters, including VaxiJen for antigenicity, ToxinPred for toxin prediction, Algpred for allergenicity, and ProtParam for sequence analysis and stability (26).

User-defined parameters, such as the desired number of vaccine sequences, serve as thresholds that are used to keep track of the number of candidates that meet all quality criteria. The algorithm exports the final multi-epitope sequence constructs once the preferred number of candidates is reached. This dynamic approach ensures the selection of vaccine sequences that meet strict standards of immunogenicity, safety, and biochemical attributes, offering researchers the most promising candidates for further development and validation. Following this, the final sequences are ranked based on the collective sum of individual rankings of antigenicity, toxicity, allergenicity, and stability in decreasing order, facilitating the identification of the optimal sequences for further analysis.

Users can evaluate their chosen sequence for several parameters, including human population coverage, molecular mimicry, proteasome cleavages, and re-analysis for epitope prediction on the newly generated sequence.

The population coverage analysis is enabled for human using the IEDB Population Coverage (27) algorithm, which gets user-selected alleles and evaluates the coverage of CTL and HTL epitopes on the vaccine sequence across regions defined by the user.

To address molecular mimicry users can conduct protein similarity searches against SwissProt (28) or metagenomic proteins (env_nr) using the BLASTp (29) algorithm. Users can determine whether the vaccine sequence shares similar domains with a protein, thereby ensuring its antigenicity and minimizing the risk of autoimmune reactions.

Furthermore, users can evaluate their preferred vaccine sequence using the NetChop 3.1 (30) module, which identifies proteasome cleavages on protein sequences. Users have the flexibility to adjust the default threshold and algorithm settings for the module. Upon execution, NetChop generates results in tabular format and a comprehensive visualization is provided, depicting the positions of epitopes and proteasome cleavages together.

Finally, users have the option to evaluate the multi-epitope vaccine sequence for the presence of the initial epitopes or new ones. Users can select the method for analysis within this module. Upon execution, the results include a data table listing new epitopes, a visual plot depicting new epitopes on the sequence, and a comparison table between the initial epitopes and the predicted ones, along with any mismatches identified between them.

RESULTS

Multi-epitope Vaccine Sequences Prediction Example

To demonstrate VaccineDesigner capabilities, we implemented an example scenario using the Atl (Bifunctional Autolysin, UniProt: P0C5Z8) and the IsdA, (Iron-regulated surface determinant protein A, UniProt: Q7A152) protein sequences from *Staphylococcus aureus* as targets. These protein sequences can be selected from the "Upload" tab panel after uploading the corresponding FASTA file for of each epitope prediction module.

B-cell epitope prediction. 123 B-cell epitopes were predicted from Atl and 136 from IsdA using Bepipred scores with a standard amino acid length of 10. The parameters used for epitope prediction were set to "Analysis 2" to obtain standard-length epitopes, with the threshold set to 0.1512, the default value. 13 of these epitopes were considered candidates for inclusion in the vaccine sequences based on their quantified toxicity, allergenicity and antigenicity measures.

CTL epitope prediction. 96 CTL epitopes from Atl and 18 from IsdA were predicted as strong or weak binders to MHC class I alleles, including *HLA-A0101, HLA-A0102, HLA-A0103, HLA-A0104, HLA-A0106, HLA-A0107, HLA-A0108* and *HLA-A0109*. These predicted epitopes underwent toxicity, allergenicity and antigenicity filters, leading to the identification of six promising candidates.

HTL epitope prediction. The number of epitopes considered strong or weak binders to MHC class II alleles, including *DBR1_0101, DBR1_0102, DBR1_0103, DBR1_0104, DBR1_0105, DBR1_0106, DBR1_0107* and *DBR1_0108* totalled 193 from Atl and 50 from IsdA. After applying the filtering step for allergenicity and toxicity, the resulting epitopes were reduced to 19.

Multi-epitope vaccine sequences. After uploading the tabular-formatted results of the candidate epitopes, the sequences were combined following the C-H-B order (CTL, HTL and B-cell). The number of B cell epitopes included in the vaccine sequence was set to 4 and the number of both CTL and HTL epitopes included was set to 3, resulting in 864 vaccine sequences. Table 1a lists the first 10 sequences that passed the antigenicity and toxicity filtering along with their properties (Table 1b).

End-to-end execution is implemented without special server-side memory and processing requirements and time-efficiently, as each building module requires about one minute of runtime with minor deviations depending on the number of input sequences.

Table 1a

Seq. ID	Sequences
1	AAYQVNSSINDYAAYVSDNKSQQTAAYYLRSHNYSYGPGPGINGEISYMKNNYQNAGPGPGMDDYMQHPGKVIKQNGPGPGNGEISY MKNNYQNAFKKSTSTTAPKTNKKDTRANQSATTKKSDNKSQQTNKKKVSDNKSQQTN
2	AAYQVNSSINDYAAYVSDNKSQQTAAYYLRSHNYSYGPGPGINGEISYMKNNYQNAGPGPGMDDYMQHPGKVIKQNGPGPGNGEISY MKNNYQNAFKKSTSTTAPKTNKKSDNKSQQTNKKKDTRANQSATTKKVSDNKSQQTN
3	AAYQVNSSINDYAAYVSDNKSQQTAAYYLRSHNYSYGPGPGINGEISYMKNNYQNAGPGPGMDDYMQHPGKVIKQNGPGPGNGEISY MKNNYQNAFKKDTRANQSATTKKSTSTTAPKTNKKSDNKSQQTNKKKVSDNKSQQTN
4	AAYQVNSSINDYAAYVSDNKSQQTAAYYLRSHNYSYGPGPGINGEISYMKNNYQNAGPGPGMDDYMQHPGKVIKQNGPGPGNGEISY MKNNYQNAFKKDTRANQSATTKKSDNKSQQTNKKKSTSTTAPKTNKKVSDNKSQQTN
5	AAYQVNSSINDYAAYVSDNKSQQTAAYYLRSHNYSYGPGPGINGEISYMKNNYQNAGPGPGMDDYMQHPGKVIKQNGPGPGNGEISY MKNNYQNAFKKDTRANQSATTKKSDNKSQQTNKKKVSDNKSQQTNKKSTSTTAPKTN

Table 1b

Seq. ID	Vaxijen Score	Toxinpred Score	Algpred Score	Instability index	GRAVY score
1	1.0539	0.245	0.262	38.62	-1.393
2	1.0527	0.245	0.262	38.62	-1.393
3	1.0408	0.245	0.262	38.62	-1.393
4	1.0443	0.245	0.262	38.62	-1.393
5	1.06	0.245	0.262	38.62	-1.393

VaccineDesigner stands out from similar platforms in several ways. Firstly, Firstly, reverse-vaccinology platforms like Vaxign2 (31) focus on predicting vaccine candidates from pathogen genomes. Our tool differs since it offers users the flexibility to analyze proteins of their preference, facilitating the prediction of immunogenic regions for tailored vaccine development. Secondly, compared to the iVAX Suite (32), we include B-cell epitope prediction and utilize a broader array of alleles, offering a more comprehensive assessment. Additionally, our platform is freely accessible and supports multi-epitope vaccine design, unlike Vacceed (33), which is limited to command-line use for automating the process of identifying vaccine candidates from thousands of protein sequences of the target pathogen. Furthermore, our integrated workflow unifies in a user-friendly interface the process of epitope vaccine development, distinguishing us from the Immune Epitope Database and Analysis Resource (IEDB) (34).

DISCUSSION

In the evolving landscape of infectious diseases, epitope-based vaccine design has emerged as a pivotal strategy, offering rationalized detection of potent immune responses. Epitope-based methods focus on the identification of antigenic determinants in pathogenic proteins, enabling the development of vaccines that reduce the risk of adverse reactions and ensure broad coverage across various pathogenic strains. VaccineDesigner serves this goal by seamlessly integrating B-cell, CTL, and HTL epitope prediction tools and quality assessment algorithms, and by generating reliable multi-epitope vaccine sequences that are further assessed for population coverage and molecular mimicry.

VaccineDesigner has a user-friendly Web interface that streamlines processes for the discovery and optimization of multi-epitope constructs and relies on a modular architecture that ensures continuous improvements toward immunogenic and safer vaccines. Therefore, as our knowledge of the complex molecular interactions evolves VaccineDesigner can both benefit by integrating new functionalities and promote incremental research through knowledge synthesis, broad usage, and adaptability in real-world applications.

DATA AVAILABILITY

VaccineDesigner is an open-source tool freely available under the academic license at https://github.com/BiolApps/VaccineDesigner (35)

REFERENCES

1. Thomas, S., and Luxon, B. A. (2013). Vaccines based on structure-based design provide protection against infectious diseases. Expert review of vaccines, 12(11), 1301–1311.

2. Webster, R. G., and Govorkova, E. A. (2014). Continuing challenges in influenza. Annals of the New York Academy of Sciences, 1323(1), 115–139.

3. Ayyagari, V. S., T C, V., K, A. P., and Srirama, K. (2022). Design of a multi-epitope-based vaccine targeting M-protein of SARS-CoV2: an immunoinformatics approach. Journal of biomolecular structure and dynamics, 40(7), 2963–2977.

4. Potocnakova, L., Bhide, M., and Pulzova, L. B. (2016). An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. Journal of immunology research, 2016, 6760830.

5. Shepherd, F. R., and McLaren, J. E. (2020). T Cell Immunity to Bacterial Pathogens: Mechanisms of Immune Control and Bacterial Evasion. International journal of molecular sciences, 21(17), 6144.

6. Zhang L. (2018). Multi-epitope vaccines: a promising strategy against tumors and viral infections. Cellular and molecular immunology, 15(2), 182–184.

7. Feng, Y., Jiang, H., Qiu, M., Liu, L., Zou, S., Li, Y., Guo, Q., Han, N., Sun, Y., Wang, K., Lu, L., Zhuang, X., Zhang, S., Chen, S., and Mo, F. (2021). Multi-Epitope Vaccine Design Using an Immunoinformatic Approach for SARS-CoV-2. Pathogens (Basel, Switzerland), 10(6), 737.

8. Yang, Z., Bogdan, P. and Nazarian, S. (2021) An in silico deep learning approach to multi-epitope vaccine design: a SARS-CoV-2 case study. Sci Rep 11, 3238.

9. Raoufi, E., Hemmati, M., Eftekhari, S., Khaksaran, K., Mahmodi, Z., Farajollahi, M. M., and Mohsenzadegan, M. (2020). Epitope Prediction by Novel Immunoinformatics Approach: A State-of-the-art Review. International journal of peptide research and therapeutics, 26(2), 1155–1163.

10. Clifford, J. N., Høie, M. H., Deleuran, S., Peters, B., Nielsen, M., and Marcatili, P. (2022). BepiPred-3.0: Improved B-cell epitope prediction using protein language models. Protein science: a publication of the Protein Society, 31(12), e4497.

11. Reynisson, B., Alvarez, B., Paul, S., Peters, B., and Nielsen, M. (2020). NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic acids research, 48(W1), W449–W454.

12. Paul, S., Sidney, J., Sette, A., and Peters, B. (2016). TepiTool: A Pipeline for Computational Prediction of T Cell Epitope Candidates. Current protocols in immunology, 114, 18.19.1–18.19.24.

13. Doytchinova, I. A., and Flower, D. R. (2007). VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics, 8, 4.

14. Sharma, N., Naorem, L. D., Jain, S., and Raghava, G. P. S. (2022). ToxinPred2: an improved method for predicting toxicity of proteins. Briefings in bioinformatics, 23(5), bbac174.

15. Sharma, N., Patiyal, S., Dhall, A., Pande, A., Arora, C., and Raghava, G. P. S. (2021). AlgPred 2.0: an improved method for predicting allergenic proteins and mapping of IgE epitopes. Briefings in bioinformatics, 22(4), bbaa294.

16. Yang, Y., Sun, W., Guo, J., Zhao, G., Sun, S., Yu, H., Guo, Y., Li, J., Jin, X., Du, L., Jiang, S., Kou, Z., and Zhou, Y. (2015). In silico design of a DNA-based HIV-1 multi-epitope vaccine for Chinese populations. Human vaccines & immunotherapeutics, 11(3), 795–805.

17. Yano, A., Onozuka, A., Asahi-Ozaki, Y., Imai, S., Hanada, N., Miwa, Y., and Nisizawa, T. (2005). An ingenious design for peptide vaccines. Vaccine, 23(17-18), 2322–2326.

18. Chen, X., Zaro, J. L., and Shen, W. C. (2013). Fusion protein linkers: property, design and functionality. Advanced drug delivery reviews, *65*(10), 1357–1369.

19. Livingston, B., Crimi, C., Newman, M., Higashimoto, Y., Appella, E., Sidney, J., and Sette, A. (2002). A rational strategy to design multiepitope immunogens based on multiple Th lymphocyte epitopes. Journal of immunology (Baltimore, Md. : 1950), 168(11), 5499–5506.

20. Li, X., Guo, L., Kong, M., Su, X., Yang, D., Zou, M., Liu, Y., and Lu, L. (2015). Design and Evaluation of a Multi-Epitope Peptide of Human Metapneumovirus. Intervirology, 58(6), 403–412.

21. Willimsky, G., Beier, C., Immisch, L., Papafotiou, G., Scheuplein, V., Goede, A., Holzhütter, H. G., Blankenstein, T., and Kloetzel, P. M. (2021). In vitro proteasome processing of neo-splicetopes does not predict their presentation in vivo. eLife, 10, e62019.

22. Díaz-Dinamarca, D. A., Salazar, M. L., Castillo, B. N., Manubens, A., Vasquez, A. E., Salazar, F., and Becker, M. I. (2022). Protein-Based Adjuvants for Vaccines as Immunomodulators of the Innate and Adaptive Immune Response: Current Knowledge, Challenges, and Future Opportunities. Pharmaceutics, 14(8), 1671.

23. Toussi, D. N., Carraway, M., Wetzler, L. M., Lewis, L. A., Liu, X., and Massari, P. (2012). The amino acid sequence of Neisseria lactamica PorB surface-exposed loops influences Toll-like receptor 2-dependent cell activation. Infection and immunity, 80(10), 3417–3428.

24. Ghaffari-Nazari, H., Tavakkol-Afshari, J., Jaafari, M. R., Tahaghoghi-Hajghorbani, S., Masoumi, E., and Jalali, S. A. (2015). Improving Multi-Epitope Long Peptide Vaccine Potency by Using a Strategy that Enhances CD4+ T Help in BALB/c Mice. PloS one, 10(11), e0142563.

25. Del Giudice G. (1994). Hsp70: a carrier molecule with built-in adjuvanticity. Experientia, 50(11-12), 1061–1066.

26. Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D., and Hochstrasser, D. F. (1999). Protein identification and analysis tools in the ExPASy server. Methods in molecular biology (Clifton, N.J.), 112, 531–552.

27. Dhanda, S. K., Mahajan, S., Paul, S., Yan, Z., Kim, H., Jespersen, M. C., Jurtz, V., Andreatta, M., Greenbaum, J. A., Marcatili, P., Sette, A., Nielsen, M., and Peters, B. (2019). IEDB-AR: immune epitope database-analysis resource in 2019. Nucleic acids research, 47(W1), W502–W506.

28. Bairoch, A., and Apweiler, R. (2000). The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. Nucleic acids research, 28(1), 45–48.

29. McGinnis, S., and Madden, T. L. (2004). BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic acids research, 32(Web Server issue), W20–W25.

30. Saxová, P., Buus, S., Brunak, S., and Keşmir, C. (2003). Predicting proteasomal cleavage sites: a comparison of available methods. International immunology, 15(7), 781–787.

31. Ong, E., Cooke, M. F., Huffman, A., Xiang, Z., Wong, M. U., Wang, H., Seetharaman, M., Valdez, N., and He, Y. (2021). Vaxign2: the second generation of the first Web-based vaccine design program using reverse vaccinology and machine learning. Nucleic acids research, 49(W1), W671–W678.

32. Moise, L., Gutierrez, A., Kibria, F., Martin, R., Tassone, R., Liu, R., Terry, F., Martin, B., and De Groot, A. S. (2015). iVAX: An integrated toolkit for the selection and optimization of

antigens and the design of epitope-driven vaccines. Human vaccines & immunotherapeutics, 11(9), 2312–2321.

33. Goodswen, S. J., Kennedy, P. J., and Ellis, J. T. (2014). Vacceed: a high-throughput in silico vaccine candidate discovery pipeline for eukaryotic pathogens based on reverse vaccinology. Bioinformatics (Oxford, England), 30(16), 2381–2383.

34. Vita, R., Mahajan, S., Overton, J. A., Dhanda, S. K., Martini, S., Cantrell, J. R., Wheeler, D. K., Sette, A., and Peters, B. (2019). The Immune Epitope Database (IEDB): 2018 update. Nucleic acids research, 47(D1), D339–D343.

35. Trygoniaris, D. and Malousi A. (2023). VaccineDesigner (Version Version3). Zenodo. https://doi.org/10.5281/zenodo.10090612

TABLE AND FIGURES LEGENDS

Table 1a. The top five multi-epitope sequences candidates that passed the filtering step of antigenicity and toxicity alongside with their respective ID.

Table 1b. The top five multi-epitope sequence candidates that passed the filtering step of antigenicity and toxicity alongside with their antigenicity, toxicity, allergenicity, GRAVY scores and instability indexes.

Figure 1. The main functional components of the streamlined process implemented by VaccineDesigner.

A. Epitope prediction module initiates with the upload of a fasta protein file, performs epitope prediction (B cell, CTL, HTL) and ends up with the evaluation and filtering of epitope sequences based on their physicochemical properties. B. Multi-epitope sequence generation module takes as input the CSV results of epitope sequences and generates vaccine sequences based on user-defined parameters. The next step is the evaluation and selection of the vaccine sequences that meet the quality criteria. Finally, the user are able to evaluate the vaccine sequence of their preference for population coverage, molecular mimicry, proteasome cleavages and re-analyse with epitope prediction modules.