Biosaintifika 16 (3) (2024): 384-393 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Computational Design of siRNA Targeting *Homo sapiens* HER2 Splice Variant mRNA: A Potential Strategy for Breast Cancer Intervention

Arli Aditya Parikesit[1,2*], Arif Nur Muhammad Ansori[2,3,4,5], Viol Dhea Kharisma[5,6]

[1]Department of Bioinformatics, School of Life Sciences, Indonesia International Institute for Life Sciences, Jakarta, Indonesia.
[2]European Virus Bioinformatics Center, Jena, Germany.
[3]Postgraduate School, Universitas Airlangga, Surabaya, Indonesia.
[4]Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India.
[5]Division of Research and Development, Jalan Tengah, Surabaya, Indonesia.
[6]Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

*Corresponding Author e-mail: arli.parikesit@i3l.ac.id

Submitted: 2024-06-14. Revised: 2024-09-08. Accepted: 2024-11-12.

Abstract. This research focuses on an innovative approach utilizing in silico methods to design small interfering RNA (siRNA) targeting the HER2 splice variant mRNA in *Homo sapiens*. HER2 is known to be overexpressed in certain types of breast cancer, contributing to tumor progression and poor prognosis. By designing siRNA molecules that can specifically bind to and degrade HER2 mRNA, this study aims to reduce HER2 protein levels, thereby hindering the growth and spread of breast cancer cells. The in-silico design process involves identifying optimal siRNA sequences that maximize target specificity and minimize off-target effects, which is crucial for potential therapeutic applications. This approach represents a promising step towards personalized medicine in the treatment of breast cancer, offering a targeted strategy to combat this variant associated with aggressive disease. The methodology comprises the RNA computational tools used for the design, the selection criteria for siRNA candidates, and the potential implications of this research in a clinical setting. The resulting outcomes are 2D and 3D siRNA designs that could potentially silence HER2 mRNA through an in-silico approach. The leads were generated using a de novo modeling approach, with no existing template available in GenBank. Moreover, it is concluded that computational tools can generate sufficiently stable 2D and 3D RNA models that could be advanced for further molecular simulation studies. The benefit of this outcome is that it facilitates better preparation for wet laboratory experiments in siRNA assays, with future implementation in vivo and clinical trial settings.

Keywords: Breast Cancer; computational tools; HER2; siRNA; mRNA

How to cite : Parikesit, A. A., Ansori, A. N. M., & Kharisma, V. D. (2024). Computational design of siRNA targeting *Homo sapiens* HER2 splice variant mRNA: A potential strategy for breast cancer intervention. *Biosaintifika: Journal of Biology & Biology Education, 16(3)*, 384-393.

DOI: http://dx.doi.org/10.15294/biosaintifika.v16i3.3685

INTRODUCTION

Breast cancer remains one of the most prevalent and devastating diseases affecting women worldwide (WHO, 2024; World Health Organization, 2021). Standard therapy for breast cancer typically involves a combination of treatments, including surgery, chemotherapy, and radiotherapy (Wang & Wu, 2023). Surgery is often the first step to remove the tumor, followed by chemotherapy to target any remaining cancer cells (Fisusi & Akala, 2019). Radiotherapy is then used to destroy any residual cancerous tissue, reducing the risk of recurrence. This multi-faceted approach aims to maximize the chances of successful treatment and long-term survival. However, these approaches are time-consuming, laborious, and expensive. Moreover, they do not specifically target the exact molecular mechanisms of breast cancer itself (Tufail et al., 2022). Some approaches have been devised to develop drugs from natural products, although clinical trials are not yet within reach (Dimarti et al., 2020; Widiyastuti et al., 2019).

The human epidermal growth factor receptor 2 (HER2) has been implicated in the pathogenesis of a subset of breast cancers characterized by aggressive growth and poor prognosis (Vranić et al., 2021). The HER2 splice variant, a messenger RNA (mRNA) transcript variant, has been identified as a potential target for therapeutic intervention due to its overexpression in certain

breast cancer cells (Swain et al., 2023, p. 20). Consequently, this research focuses on invoking monoclonal antibodies and dendritic cells to inhibit the HER2 protein (Costa & Czerniecki, 2020). However, these approaches have complex manufacturing processes es (Ain et al., 2023). In this regard, RNA interference (RNAi) technology has opened new avenues for cancer treatment (Rajan et al., 2020). Small interfering RNA (siRNA) molecules, a cornerstone of RNAi, can specifically downregulate the expression of target genes. siRNA is a type of RNA interference (RNAi) tool that can bind to complementary mRNA sequences and induce their degradation via the RNA-induced silencing complex (RISC) (J. Zhang et al., 2023). The design of siRNA molecules targeting the HER2 splice variant mRNA holds promise as a novel approach to mitigate the progression of HER2-positive breast cancer (Y. Zhang, 2021, p. 202). This rational approach works by deterring HER2 mRNA, preventing it from being expressed as a protein in the cell.

In silico methods offer a powerful platform for the rational design of siRNA, enabling the identification of the most efficacious siRNA sequences with minimal off-target effects (Valdés & Miller, 2019). Our approach delves into the in silico design of siRNA targeting the *Homo sapiens* HER2 splice variant mRNA, exploring its potential as a deterrence strategy for breast cancer. Through computational modeling and analysis, we aim to elucidate the therapeutic potential of siRNA molecules in silencing aberrant HER2 expression, thereby contributing to the arsenal of targeted therapies against breast cancer (Ali Zaidi et al., 2023).

This project proposes to design siRNA molecules that can specifically target and silence both the wild-type and splice variants of HER2 mRNA, thereby inhibiting HER2 protein expression and downstream signaling. By reducing HER2 protein levels, siRNA could potentially induce apoptosis and inhibit the growth and metastasis of HER2-positive breast cancer cells. This approach is supported by studies demonstrating the effectiveness of siRNA in targeting HER2 mRNA, leading to decreased tumor viability and proliferation. For example, engineered exosomes have been utilized for the targeted transfer of siRNA to HER2-positive breast cancer cells, demonstrating significant down-regulation of gene expression and potential therapeutic effects (Limoni et al., 2019). Furthermore, the development of targeted therapeutic options continues to evolve, offering promising strategies for managing HER2-positive breast cancer (Wang & Xu, 2019). The specificity and efficacy of these siRNA molecules are critical for ensuring successful gene silencing without affecting non-targeted cells, thus minimizing potential side effects and enhancing the therapeutic index.

The research benefits and objectives of this manuscript include the computational design of siRNAs targeting the mRNA splice variants of the HER2 gene in breast cancer. This work is envisioned to yield potent siRNA sequences that selectively silence the HER2 splice variant and potentially overcome resistance to therapies. In this context, this work enhances the field of RNA interference-based cancer therapy by computationally optimizing siRNA design while considering off-target effects and target accessibility. Thus, it can enable more personalized and effective treatment for patients with this form of HER2-positive breast cancer, allowing for a better prognosis with new insights into disease biology and mechanisms leading to drug resistance. The study depicted here demonstrates how computational biology, along with future experimental validation, can accelerate the nucleic acid therapy development process and reduce the time and resources needed for siRNA screening. This ultimately furthers the exploration of alternatives to antibodies and small-molecule inhibitors in targeted cancer therapy, introducing new methods for breast cancer intervention and patient care.

METHODS

The existing updated pipeline (Mursyidin et al., 2021; Parikesit et al., 2022; Valeska & Parikesit, 2022) was utilized. Generated data were uploaded to the ZENODO website (https://zenodo.org) as a data repository (Parikesit, 2024).

Data Retrieval and Pairwise Alignment

Two entries for "HER2 *Homo sapiens*" in the NCBI GenBank were found. The target genes for the siRNA were GenBank ID no. MW358920.1 (https://www.ncbi.nlm.nih.gov/nuccore/MW358920.1) and MW423435.1 (https://www.ncbi.nlm.nih.gov/nuccore/MW423435.1) (Sayers et al., 2022). Both entries were downloaded in FASTA format. A pairwise alignment operation was then executed in MEGA11 to determine the conserved region. The alignment was conducted using the

CLUSTALW method. Additionally, gap opening and extension penalty parameters were set to their default values (Tamura et al., 2021, p. 202).

siRNA Design as HER2 mRNA Repressor

The RNAxs software was accessed at this link: http://rna.tbi.univie.ac.at/cgi-bin/RNAxs/RNAxs.cgi. The conserved region sequence was input in the query box, with both design and output options set to default values. The expected output was the FASTA format of the siRNA design (Lorenz & Stadler, 2021).

Conserved Structures of HER2 mRNA and siRNA

The RNAalifold software was accessed at this link: http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAalifold.cgi. The RNAalifold version, fold algorithms, and basic options were all set to default values. Additionally, the conserved sequences of both mRNA and siRNA were extracted for interaction studies (Varenyk et al., 2023).

Predicted 2D Structures of Both mRNA and siRNA Conserved Sequences

The RNAfold software was accessed at this link: http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi. Both the mRNA and siRNA conserved sequences were inserted into the designated query box (Yao et al., 2023). Additionally, the folding constraints, fold algorithms, basic options, dangling end options, energy parameters, modified base, and SHAPE reactivity data parameters were all set to default values.

3D Structure Generation

The iFOLDRNAv2 software was accessed at this link: https://dokhlab.med.psu.edu/ifoldrna (Wang & Dokholyan, 2022). Logging in to the website with the registered profile was required. The "RNA Modeling" task option was chosen, with all parameters left at default values. The RNA sequence in Vienna format annotation and secondary structure were inserted into the designated query box. The output structures were visualized with UCSF ChimeraX version 1.7.1 (Pettersen et al., 2021).

3D Structure Validation

Structure validation was conducted using the MolProbity server at http://molprobity.biochem.duke.edu/index.php. The resulting PDB models were input into the query box, with all parameters left at default values. Various parameters were checked for necessary corrections, including sugar puckers, bad backbone conformations, bad bonds, and bad angles (Molprobity, 2021; Williams et al., 2018). Finally, the AVOGADRO molecular editor software version 2.0 was used to protonate and minimize the free energy of the structures, invoking default parameters (Snyder & Kucukkal, 2021).

RESULTS AND DISCUSSION

Data Retrieval and Pairwise Alignment

As observed in Figure 1, neither sequence was extensively conserved. However, they are plausibly conserved and can be extracted as the basis for the siRNA design. Although both mRNAs express different protein isoforms, the conserved target would be directed to the mRNA backbone. This strategy leverages the conserved regions of the mRNA to design siRNAs that are likely to be effective across various isoforms, maximizing the potential impact of the therapy.

siRNA Design as HER2 mRNA Repressor

The RNAxs software required less than 10 minutes of processing time for each sequence. As presented in Figure 2, all hits were BLAST-validated to HER2 genes. As indicated in Figure 2a, the HER2 I siRNA sequence is TAAAACTAAACCCCCACCC. Furthermore, as indicated in Figure 2b, the HER2 II siRNA sequence is TTGATGGGGCAAGGCTGAC. The Roman numeral designation refers to the first and second rank of the outcomes in the RNAxs software based on the plausibility of the design. Additionally, as presented in Figure 3, neither sequence is fully conserved, meaning that each targets different positions in the HER2 mRNA.

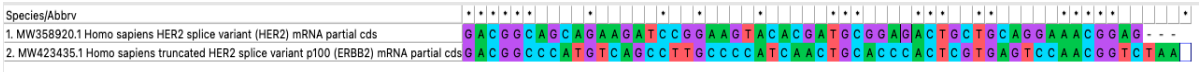

Figure 1. Annotated mRNAs of HER2 splice variants with MEGA11 software

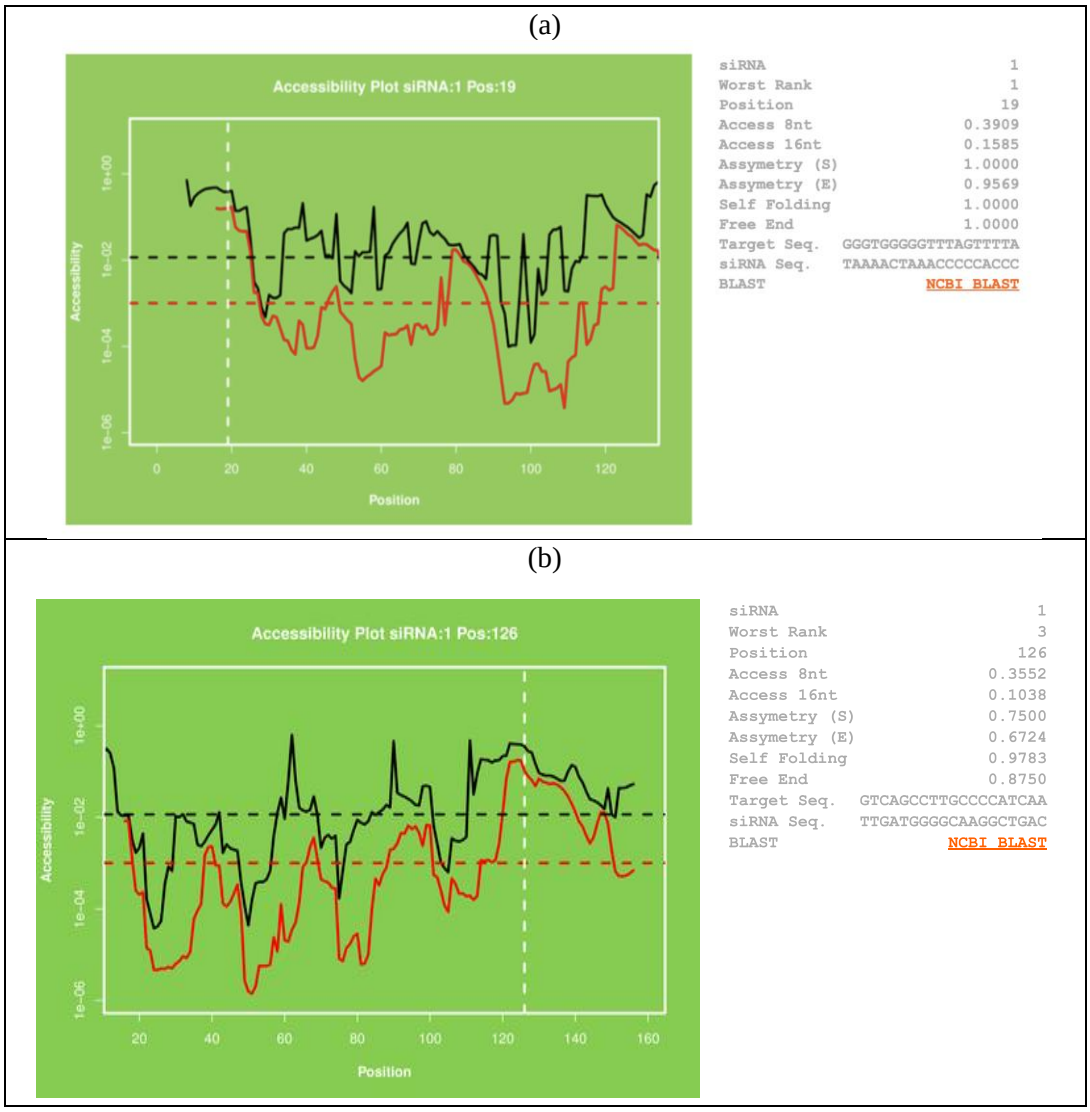

Figure 2. a) mRNA HER2 I sequence b) mRNA HER2 II sequence

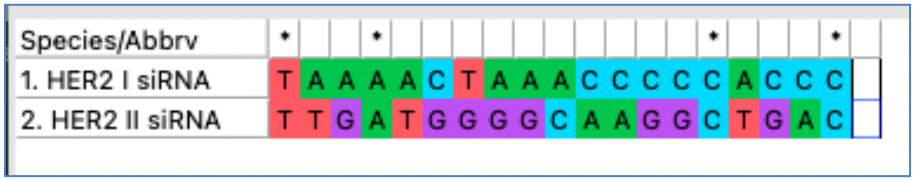

Figure 3. Sequence alignment of mRNA HER2 I and HER2 II sequences with MEGA11 software

Conserved Structure of HER2 mRNA and siRNA

As indicated in the RNAalifold outcomes in Figure 4, the mRNA structures of both HER2 variant genes are sufficiently conserved and plausible. This is evidenced by the minimum free energy (MFE) value of -47.19 kcal/mol, reflecting the negative free energy outcome of a spontaneous reaction (Leppek et al., 2022). Additionally, the RNAalifold outcomes in Figure 5 demonstrate that the conserved siRNA structure is also plausible. The structural conservation is further supported by the alignment of multiple sequences, revealing a high degree of similarity in the siRNA regions across different species. This conservation suggests a critical role for these structures in gene regulation and RNA interference mechanisms. Although the MFE value for the siRNA structure is not explicitly stated, it likely indicates a stable and functionally relevant conformation. These findings underscore the utility of RNAalifold in identifying and validating conserved RNA structures crucial for biological processes (Rivas, 2020).

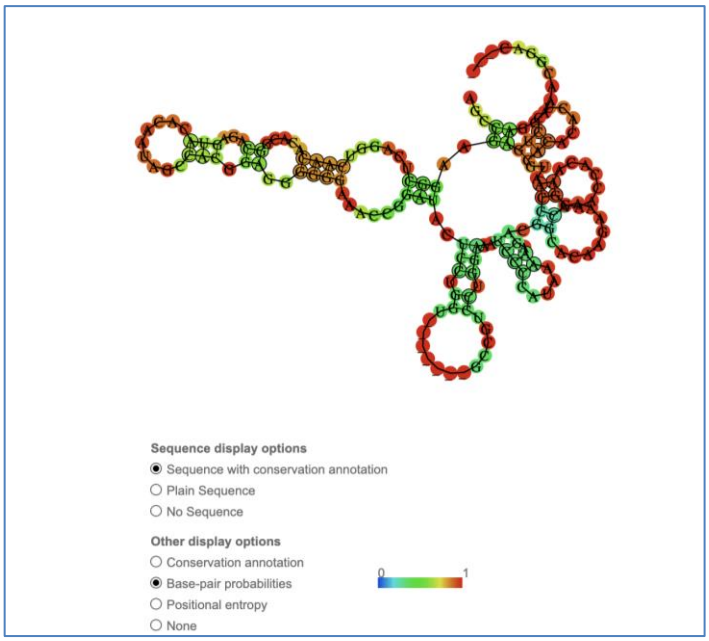

Figure 4. The conserved structure of HER2 mRNA. The legend indicates that the color gradation of base-pair probabilities ranges from 0 to 1. A value of 0 means that the sequence is not fully conserved, while a value of 1 means it is highly conserved.

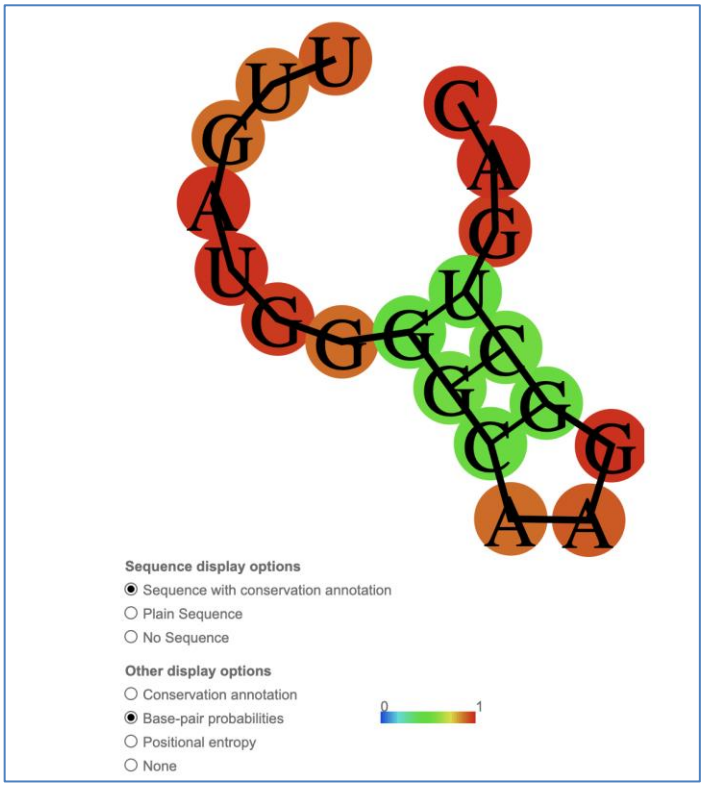

Figure 5. The conserved structure of HER2 siRNA. The legend indicates that the color gradation of base-pair probabilities ranges from 0 to 1. A value of 0 means that the sequence is not fully conserved, while a value of 1 means it is highly conserved.

The plausibility of the conserved structures of both mRNA and siRNA allows us to observe the interactions of both chemical leads. The conserved structures represent all possible structures generated in the cell based on the existing GenBank annotations. The conserved sequences listed in Table 1 will be used for further structure prediction.

Table 1. Conserved sequences of both mRNA and siRNA of the HER2 Gene

No.	Sequence in FASTA Format
1.	> Conserved sequence of mRNA HER2 AGCCGAAGGCUCAGGUCAACACACGGCAGAGUACACAAUAGCCACGGAGGGGGGAAACC GGAUACUCCUGGU_____GCCGUCCUGGAAAUCCCCAUAAAAAGACGGCACAAGAAAAC CCGGAACCACAAAAUGCACACACUCCUGAAGCAAACGGAC___
2.	> Conserved sequence of siRNA HER2 UUGAUGGGGCAAGGCUGAC

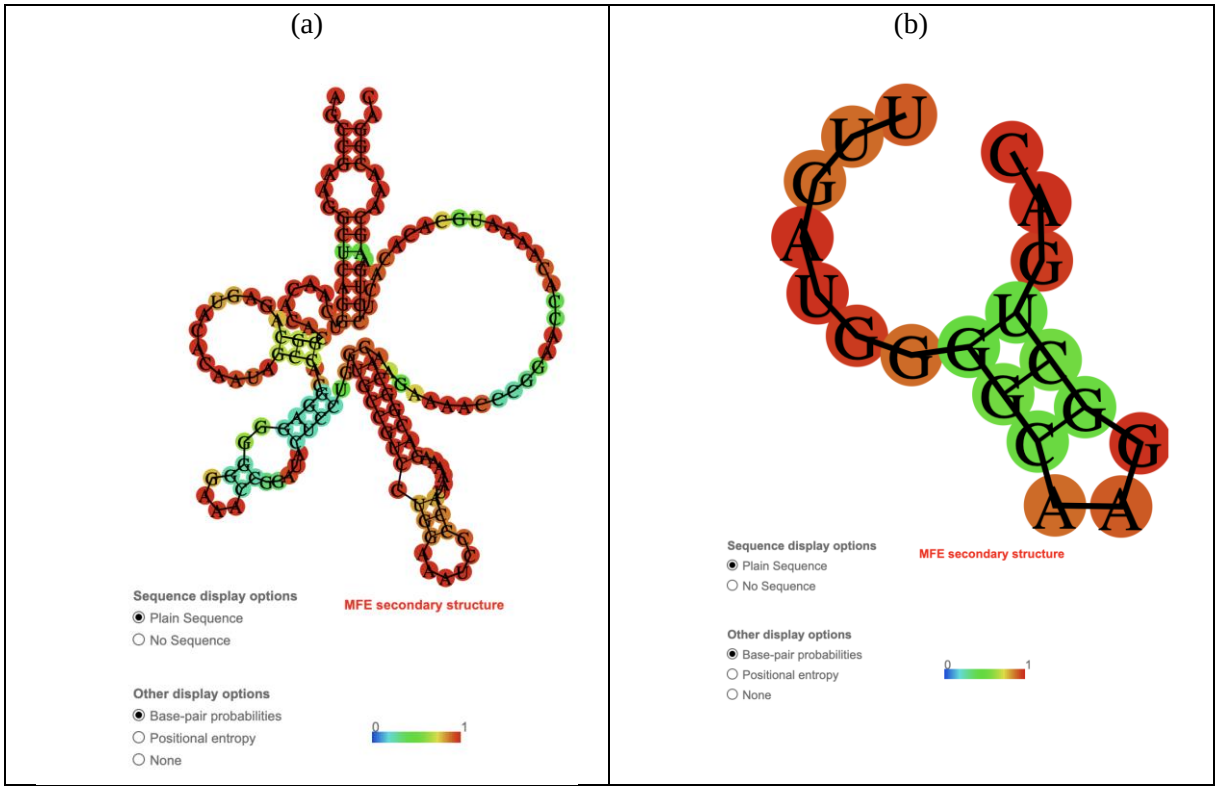

Figure 6. a) HER2 mRNA b) HER2 siRNA predicted structures. The legend indicates that the color gradation of base-pair probabilities ranges from 0 to 1. A value of 0 means that the sequence is not fully conserved, while a value of 1 means it is highly conserved.

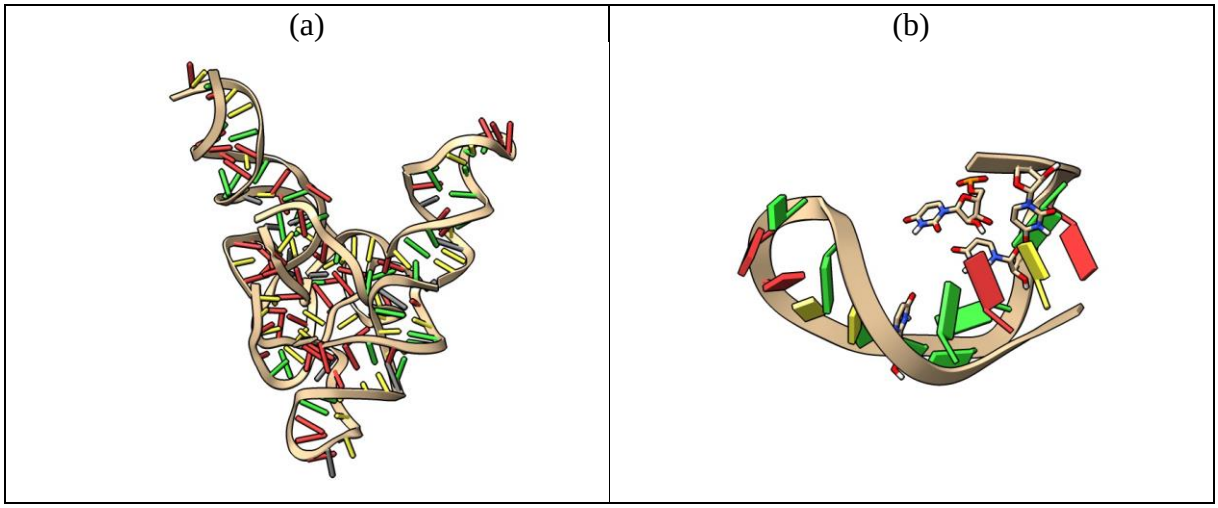

Figure 7. a) mRNA HER2 b) siRNA HER2 Predicted 3D structures

Table 2. The outcomes of the molprobity program. The red column and row are designated to values below the optimized threshold.

Indicator	Parameter	siRNA 3D predicted structure model		mRNA 3D predicted structure model		Notes
Nucleic Acid Geometry	Probably wrong sugar puckers:	3	15.79%	10	6.54%	Goal: 0
	Bad bonds:	15/460	3.26%	139/3682	3.78%	Goal: 0%
	Bad angles:	33/714	4.62%	308/5739	5.37%	Goal: < 0.1%
Additional validations	Chiral volume outliers	0/95		0/765		

Predicted 2D Structures of Both mRNA and siRNA Conserved Sequences

The RNAfold outcomes for the conserved sequences in Table 1 can be observed in Figure 6. The HER2 mRNA 2D structure was plausible, with an MFE value of -37.90 kcal/mol, indicating negative free energy and a spontaneous reaction (Figure 6a). Furthermore, the HER2 siRNA 2D structure was also plausible, with an MFE value of -0.40 kcal/mol, similarly indicating negative free energy and a spontaneous reaction (Figure 6b). These 2D structures were prepared to generate the 3D RNA models.

3D Structure Generation

The outcomes from the iFOLDRNAv2 software are presented in Figure 7. All structures were selected from the best annotations in their respective clusters, ensuring the reliability of structural predictions, as each cluster represents a set of similar structures grouped based on annotation quality. The iFOLDRNAv2 software uses experimental constraints such as base-pairing and nucleotide solvent accessibility to enhance RNA tertiary structure prediction accuracy. For instance, base-pairing information is derived from sequence covariation analysis or chemical probing techniques, while solvent accessibility is inferred using data from hydroxyl radical probing (HRP) experiments (Solayman et al., 2022). These integrated approaches allow for precise RNA structure predictions, even for sequences a few hundred nucleotides long.

3D Structure Validation

As presented in Table 2, critical parameters required for structural validation have not yet reached the optimal values specified in the "Notes" column. Parameters highlighted in red are leads for a further optimization process to achieve ideal pipeline conditions. This is primarily because RNA molecules are more prone to local structural issues than proteins, which increases steric effects from bases, phosphates, and the sugar-phosphate backbone, contributing to this tendency (Prisant et al., 2020). Additionally, as presented in Figure 7, RNA's helical structure can fold back on itself, creating a complex 3D structure similar to tRNA. Predicting RNA structure based on sequence knowledge alone can lead to significant computational complexity and increase the risk of suboptimal modeling solutions (Xiong et al., 2021).

Biological Significance of the Computational Results

The HER2 siRNA primarily degrades cancerous cells through the specific targeting of the HER2 gene, which is over-expressed in various types of breast cancer. The 2D and 3D siRNA structures designed for targeting HER2 have been optimized for high efficacy in gene silencing and effective delivery to cancer cells. Since siRNA molecules are typically designed as short, double-stranded RNA sequences in 2D structures, they are more easily taken up by cells and incorporated into RISC multiprotein complex. The 3D structures, when formed in multicellular spheroids, more closely mimic the conditions of in vivo tumors, allowing a better estimation of how siRNA might act in real tumors (Jarman et al., 2019). These 3D structures offer promising architectures that can enhance penetration and distribution in tumor-like environments, creating a more effective siRNA design. Locally administered nanoparticle platforms with systemic HER2 siRNA have demonstrated a high degree of growth inhibition in trastuzumab-resistant tumors, exemplifying this approach’s potential to overcome drug resistance (Y. Zhang, 2021). Additionally, the combination of HER2 siRNA with chemotherapeutic agents like doxorubicin has demonstrated promise in simultaneously inhibiting the expression of the target gene and

producing an anti-cancer effect (Ngamcherdtrakul & Yantasee, 2020; Subhan & Torchilin, 2024).

CONCLUSION

In conclusion, the in-silico design of siRNA targeting the HER2 splice variant mRNA was successfully conducted with a computational approach. It presents a promising therapeutic strategy for breast cancer prevention. The design of the 2D and 3D models of both siRNA and mRNA has demonstrated that the complex nature of the HER2 genes can be annotated accordingly. However, further optimization of the structures is needed. The next step of this research will involve conducting finer-grained molecular simulation studies, particularly using molecular docking and dynamics methods with high fidelity time and space complexities. Future studies are encouraged to explore the full therapeutic potential of siRNA in managing complex diseases, especially in in vitro and in vivo wet laboratory settings.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Research and Community Service of the Indonesia International Institute for Life Sciences (LPPM i3L) for their heartfelt support. They deployed Grammarly Premium to edit, revise, and proofread the manuscript. All computed data have been deposited in the ZENODO repository.

REFERENCES

Ali Zaidi, S. S., Fatima, F., Ali Zaidi, S. A., Zhou, D., Deng, W., & Liu, S. (2023). Engineering siRNA therapeutics: Challenges and strategies. *Journal of Nanobiotechnology*, *21*(1), 381. https://doi.org/10.1186/s12951-023-02147-z

Costa, R. L. B., & Czerniecki, B. J. (2020). Clinical development of immunotherapies for HER2+ breast cancer: A review of HER2-directed monoclonal antibodies and beyond. *Npj Breast Cancer*, *6*(1), 1–11. https://doi.org/10.1038/s41523-020-0153-3

Dimarti, S. C., Susilaningsih, N., & Yuniati, R. (2020). Phycocyanin from Spirulina platensis Induces Cytotoxicity and Apoptosis in T47D Cells. *Biosaintifika: Journal of Biology & Biology Education*, *12*(1), Article 1. https://doi.org/10.15294/biosaintifika.v12i1.22992

Fisusi, F. A., & Akala, E. O. (2019). Drug Combinations in Breast Cancer Therapy. *Pharmaceutical Nanotechnology*, *7*(3), 3–23. https://doi.org/10.2174/2211738507666190122111224

Leppek, K., Byeon, G. W., Kladwang, W., Wayment-Steele, H. K., Kerr, C. H., Xu, A. F., Kim, D. S., Topkar, V. V., Choe, C., Rothschild, D., Tiu, G. C., Wellington-Oguri, R., Fujii, K., Sharma, E., Watkins, A. M., Nicol, J. J., Romano, J., Tunguz, B., Diaz, F., … Das, R. (2022). Combinatorial optimization of mRNA structure, stability, and translation for RNA-based therapeutics. *Nature Communications*, *13*(1), 1536. https://doi.org/10.1038/s41467-022-28776-w

Limoni, S. K., Moghadam, M. F., Moazzeni, S. M., Gomari, H., & Salimi, F. (2019). Engineered Exosomes for Targeted Transfer of siRNA to HER2 Positive Breast Cancer Cells. *Applied Biochemistry and Biotechnology*, *187*(1), 352–364. https://doi.org/10.1007/s12010-018-2813-4

Lorenz, R., & Stadler, P. F. (2021). RNA Secondary Structures with Limited Base Pair Span: Exact Backtracking and an Application. *Genes*, *12*(1), Article 1. https://doi.org/10.3390/genes12010014

Molprobity. (2021). *Molprobity Legend for Structural Validation*. http://molprobity.biochem.duke.edu/help/validation_options/summary_table_guide.html

Mursyidin, D. H., Ahyar, G. M. Z., Saputra, A. W., & Hidayat, A. (2021). Genetic Diversity and Relationships of Phalaenopsis Based on the rbcL and trnL-F Markers: In Silico Approach. *Biosaintifika: Journal of Biology & Biology Education*, *13*(2), Article 2. https://doi.org/10.15294/biosaintifika.v13i2.29904

Ngamcherdtrakul, W., & Yantasee, W. (2020). Erratum to “siRNA therapeutics for breast cancer: Recent efforts in targeting metastasis, drug resistance, and immune evasion” [Translational Research 214C (2019) 105-120]. *Translational Research*, *217*, 77. https://doi.org/10.1016/j.trsl.2020.01.004

Parikesit, A. A. (2024). *HER2 RNA Molecular Simulation Data* [Dataset]. Zenodo. https://doi.org/10.5281/zenodo.10973054

Parikesit, A. A., Ansori, A. N. M., & Kharisma, V. D. (2022). A Computational Design of siRNA in SARS-CoV-2 Spike Glycoprotein Gene and Its Binding Capability toward mRNA. *Indonesian Journal of Chemistry*, *22*(5), 1163. https://doi.org/10.22146/ijc.68415

Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., Morris,

J. H., & Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Science*, *30*(1), 70–82. https://doi.org/10.1002/PRO.3943

Prisant, M. G., Williams, C. J., Chen, V. B., Richardson, J. S., & Richardson, D. C. (2020). New tools in MolProbity validation: CaBLAM for CryoEM backbone, UnDowser to rethink "waters," and NGL Viewer to recapture online 3D graphics. *Protein Science*, *29*(1), 315–329. https://doi.org/10.1002/pro.3786

Rajan, S. S., Ludwig, K. R., Hall, K. L., Jones, T. L., & Caplen, N. J. (2020). Cancer biology functional genomics: From small RNAs to big dreams. *Molecular Carcinogenesis*, *59*(12), 1343. https://doi.org/10.1002/mc.23260

Rivas, E. (2020). RNA structure prediction using positive and negative evolutionary information. *PLOS Computational Biology*, *16*(10), e1008387. https://doi.org/10.1371/journal.pcbi.1008387

Sayers, E. W., Cavanaugh, M., Clark, K., Pruitt, K. D., Schoch, C. L., Sherry, S. T., & Karsch-Mizrachi, I. (2022). GenBank. *Nucleic Acids Research*, *50*(D1), D161–D164. https://doi.org/10.1093/nar/gkab1135

Snyder, H. D., & Kucukkal, T. G. (2021). Computational Chemistry Activities with Avogadro and ORCA. *Journal of Chemical Education*, *98*(4), 1335–1341. https://doi.org/10.1021/acs.jchemed.0c00959

Solayman, M., Litfin, T., Zhou, Y., & Zhan, J. (2022). High-throughput mapping of RNA solvent accessibility at the single-nucleotide resolution by RtcB ligation between a fixed 5′-OH-end linker and unique 3′-P-end fragments from hydroxyl radical cleavage. *RNA Biology*, *19*(1), 1179–1189. https://doi.org/10.1080/15476286.2022.2145098

Subhan, M. A., & Torchilin, V. P. (2024). Advances in siRNA Drug Delivery Strategies for Targeted TNBC Therapy. *Bioengineering*, *11*(8), Article 8. https://doi.org/10.3390/bioengineering11080830

Swain, S. M., Shastry, M., & Hamilton, E. (2023). Targeting HER2-positive breast cancer: Advances and future directions. *Nature Reviews Drug Discovery*, *22*(2), 101–126. https://doi.org/10.1038/s41573-022-00579-0

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tufail, M., Cui, J., & Wu, C. (2022). Breast cancer: Molecular mechanisms of underlying resistance and therapeutic approaches. *American Journal of Cancer Research*, *12*(7), 2920–2949. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9360230/

Valdés, J. J., & Miller, A. D. (2019). New opportunities for designing effective small interfering RNAs. *Scientific Reports*, *9*(1), 16146. https://doi.org/10.1038/s41598-019-52303-5

Valeska, M. D., & Parikesit, A. A. (2022). Determination of 3D Structure and Molecular Interaction for mir- 135b and Its Silencer as Triple Negative Breast Cancer (TNBC) Biomarkers. *Berkala Penelitian Hayati*, *28*(1), 1–7. https://doi.org/10.23869/bphjbr.28.1.202210

Varenyk, Y., Spicher, T., Hofacker, I. L., & Lorenz, R. (2023). Modified RNAs and predictions with the ViennaRNA Package. *Bioinformatics*, *39*(11), btad696. https://doi.org/10.1093/bioinformatics/btad696

Vranić, S., Bešlija, S., & Gatalica, Z. (2021). Targeting HER2 expression in cancer: New drugs and new indications. *Bosnian Journal of Basic Medical Sciences*, *21*(1), 1–4. https://doi.org/10.17305/bjbms.2020.4908

Wang, J., & Dokholyan, N. V. (2022). Application of RNA Tertiary Structure Prediction Tool iFoldRNA in RNA Nanotechnology. In *RNA Nanotechnology and Therapeutics* (2nd ed.). CRC Press.

Wang, J., & Wu, S.-G. (2023). Breast Cancer: An Overview of Current Therapeutic Strategies, Challenge, and Perspectives. *Breast Cancer : Targets and Therapy*, *15*, 721–730. https://doi.org/10.2147/BCTT.S432526

Wang, J., & Xu, B. (2019). Targeted therapeutic options and future perspectives for HER2-positive breast cancer. *Signal Transduction and Targeted Therapy*, *4*(1), 1–22. https://doi.org/10.1038/s41392-019-0069-2

WHO. (2024). WHO | Breast cancer: Prevention and control. In *WHO*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/breast-cancer

Widiyastuti, Y., Sholikhah, I. Y. M., & Haryanti, S. (2019). Phytochemical and Cytotoxic Evaluation of Krangean Fruits Extracts Against HeLa, MCF-7, and HepG2 Cancer Cell Line. *Biosaintifika: Journal of Biology & Biology Education*, *11*(3), Article 3. https://doi.org/10.15294/biosaintifika.v11i3.18073

Williams, C. J., Headd, J. J., Moriarty, N. W., Prisant, M. G., Videau, L. L., Deis, L. N., Verma, V., Keedy, D. A., Hintze, B. J., Chen, V. B., Jain, S., Lewis, S. M., Arendall, W. B., Snoeyink, J., Adams, P. D., Lovell, S. C., Richardson, J. S., & Richardson, D. C. (2018). MolProbity: More and better reference data for improved all-atom structure validation. *Protein Science*, *27*(1), 293–315. https://doi.org/10.1002/pro.3330

World Health Organization. (2021). WHO | Breast cancer. In *World Health Organization (WHO)*. WHO Expert Panel. https://www.who.int/news/item/03-02-2021-breast-cancer-now-most-common-form-of-cancer-who-taking-action

Xiong, P., Wu, R., Zhan, J., & Zhou, Y. (2021). Pairing a high-resolution statistical potential with a nucleobase-centric sampling algorithm for improving RNA model refinement. *Nature Communications*, *12*(1), 2777. https://doi.org/10.1038/s41467-021-23100-4

Yao, H.-T., Lorenz, R., Hofacker, I. L., & Stadler, P. F. (2023). Mono-valent salt corrections for RNA secondary structures in the ViennaRNA package. *Algorithms for Molecular Biology*, *18*(1), 8. https://doi.org/10.1186/s13015-023-00236-0

Zhang, J., Chen, B., Gan, C., Sun, H., Zhang, J., & Feng, L. (2023). A Comprehensive Review of Small Interfering RNAs (siRNAs): Mechanism, Therapeutic Targets, and Delivery Strategies for Cancer Therapy. *International Journal of Nanomedicine*, *18*, 7605–7635. https://doi.org/10.2147/IJN.S436038

Zhang, Y. (2021). The root cause of drug resistance in HER2-positive breast cancer and the therapeutic approaches to overcoming the resistance. *Pharmacology & Therapeutics*, *218*, 107677. https://doi.org/10.1016/j.pharmthera.2020.107677