AI-Augmented Computational Modeling of Bispecific antibody targeting B7H4+ cancer cells and CD3e+ CAR T-Cells for Targeted Therapy in Solid Tumor

RISHI NAIR[1,2] AND GAURAV SHARMA[2]

[1]Blacksburg High School, VA, Blacksburg
[2]Eigen Sciences, NC, Apex

Compiled Apr, 2025

A solid tumor is an abnormal mass of uncontrolled cell growth, typically originating in the breast, lung, or prostate. Its physiological features make treatment by traditional methods difficult. In Solid tumors, the B7H4 receptor is overexpressed on the surface of the cancer cells, i.e., the number of receptors on cancer cells increases. This makes them an essential target for cancer diagnosis and targeted therapy. DuoBody is a bispecific antibody with two halves of antibodies designed to target two specific receptors and enhance therapeutic effects by bringing T cells to cancer cells, resulting in cancer cell apoptosis by the T cell. In this research, I am working on the B7H4 receptor, which is highly expressed in solid tumors, and the CD3e receptor, which plays a role in activating T-cell response. We hypothesize that these DuoBody antibodies can be used to target the B7H4+ solid cancer cells by inducing the CAR T-cells toward the cancer cells. In the current research, we have performed computational modeling to design DuoBodies antibodies targeting cancer (B7H4 receptor) and CAR T cells (CD3e receptor). Initially, we got the 3D structures of the receptors by using the AlphaFold 3 web server. The 3D structure of antibodies was downloaded from the protein data bank. The downloaded antibodies were docked on the predicted receptor structures utilizing the HDOCK2.0 software to understand the antibodies binding interaction and affinity. The docking results were validated using the graph neural network (GNN). The antibodies were selected based on visual inspection, binding energy, and hydrogen bond interactions of the output obtained from the molecular docking simulations. The Binding energies calculated by the PRODIGY software showed that antibodies 4a6y and 2uyl strongly bond to the CD3e receptor and that 1il1 and 1f8t strongly bond to the B7H4 receptor. This research can be used in pharmaceutical drug development to engineer Duobodys targeting cancer cells.

1. INTRODUCTION

Solid tumors are abnormal masses of tissue that form in organs or tissues, typically composed of cancerous or non-cancerous cells, as opposed to blood-based cancers like leukemia.(1) Cancerous solid tumors are the uncontrolled growth of cell tissues and can be either benign or malignant.(1) Specifically, solid tumors are solid and don't have any cysts or liquid areas.(1) They are named for the types of cells they are made of (Ex, Bone, kidney, liver, and lung cancer).(2) Solid tumors create a lump as they expand and are identified by screenings (mammograms, colonoscopies), biopsies, or blood tests(searching for tumor markers).(2) Solid tumors can spread to other parts of the body(metastasize).(3) Solid tumors are treated by surgery, radiation therapy, and chemotherapy, depending on the stage/type of the tumor.(4) Detecting a solid tumor early significantly improves the survival rate of the patient.(4) B7H4 is a receptor highly expressed in various cell tumor types and is associated with tumor aggressiveness and lower survival rates.(5) B7H4 is identified as a therapeutic target for the treatment of cancers.(5) B7H4 negatively affects the immune response of T cells and inhibits their cell cycle, playing an essential role in tumor growth.(5)

Antibodies are proteins in the bloodstream designed to discover antigens and trigger chemical reactions in the body to remove the antigens.(6) Antibodies are Y-shaped molecules with two antigen-binding sites at the two tips of the Y-shape.(6) DuoBody is a platform for the development of bi-specific antibodies.(7) DuoBodies consist of two halves of different antibodies and are designed to bind to two specific targets, triggering more of a particular pathway of chemicals.(8) The dual-targeting prop-

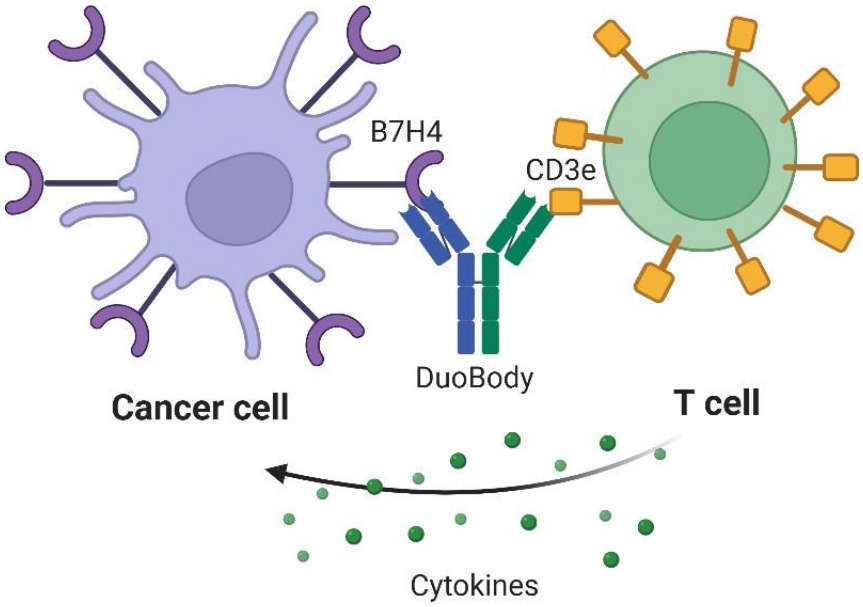

Fig. 1. Diagram of DuoBody binding to CD3e receptor on T Cell and B7H4 receptor on Cancer cell. The green half of the Duo-Body represents an antibody designed explicitly to bind to the CD3e receptor on T cells. The blue half of the DuoBody represents an antibody specifically designed to bind to the B7H4 receptor on the cancer cell.

erties of DuoBody enhance the therapeutic potential, allowing the development of specific treatments for various diseases.(8) The schematic of the bispecific antibodies is shown in Figure 1.

Molecular docking is a computer program that predicts how molecules will interact with each other.(9) In this scenario, molecular docking predicts how a ligand binds to the receptor.(9) Molecular docking works by running a search algorithm that generates possible binding models by finding each orientation of the ligand that fits into the receptor's binding area.(9) Then, the potential binding complexes are ranked based on the strength of interactions like hydrogen bonds and van der Waals forces.(10) Molecular docking is used in pharmacology, specifically in structure-based drug design and drug effects calculation.(9)

Koopman et al. have designed bispecific antibodies targeting CD3e receptors present on the surface of T cells and B7H4 located on the solid tumor cancer cell.(11) This opens up the opportunity to develop T cell-mediated solid tumor treatment. We hypothesize that these DuoBody antibodies can be used to target the B7H4+ solid cancer cells by inducing the CAR T-cells toward the cancer cells. In this research work, we have performed computational simulations to identify variable regions of antibodies specific to the two receptors. Our results suggest that 1IL1 against B7H4 and 4A6Y against CD3e receptor were selected. This research can be used in pharmaceutical drug development to engineer Duobody targeting cancer cells.

2. RESULTS

Receptor Structure Prediction: To start the research, we first obtained the 3D structure of the receptor using AlphaFold 3. It is an AI model developed by Google DeepMind and Isomorphic Labs that accurately predicts the structure of proteins, DNA, RNA, and ligands. These receptors are present on the surface of the cell and have three regions: i.e., an ectodomain (outside the cell), an endodomain (inside the cell), and a transdomain (across plasma membrane), Figure 2. To understand the surface binding properties of these receptors, we used a graph neural network analysis method called GrASP. GrASP is a graph neural network tool used to accurately find the optimal binding sight for the ligand to the receptor. Electrostatic surface potential is a three-dimensional map showing a molecule's charged regions. ESP is used in drug design to optimize interactions between proteins and ligands.

Molecular Docking Simulation: In the next step, the receptor antibody molecular docking simulations were performed using the HDOCK 2.0 web server to get the receptor-antibody complex structure.

Table 1: Receptor-antibody binding energy calculations: the binding energy is the strength of the receptors and antibodies' interaction. Antibodies with higher binding energy will be selected.

	Binding energy (kcal/mol)	
Antibodies name	**B7H4**	**CD3e**
1a5f	-13.9	-10.7
1b4j	-12.6	-12.9
1fl5	-13.5	-14.0
1il1	-13.7	-14.0
1iqw	-12.8	-14.0
1nlb	-12.9	-11.6
2hrp	-12.3	-12.0
4a6y	-11.1	-16.1
1f8t	-17.1	-14.4
2uyl	-13.6	-15.9

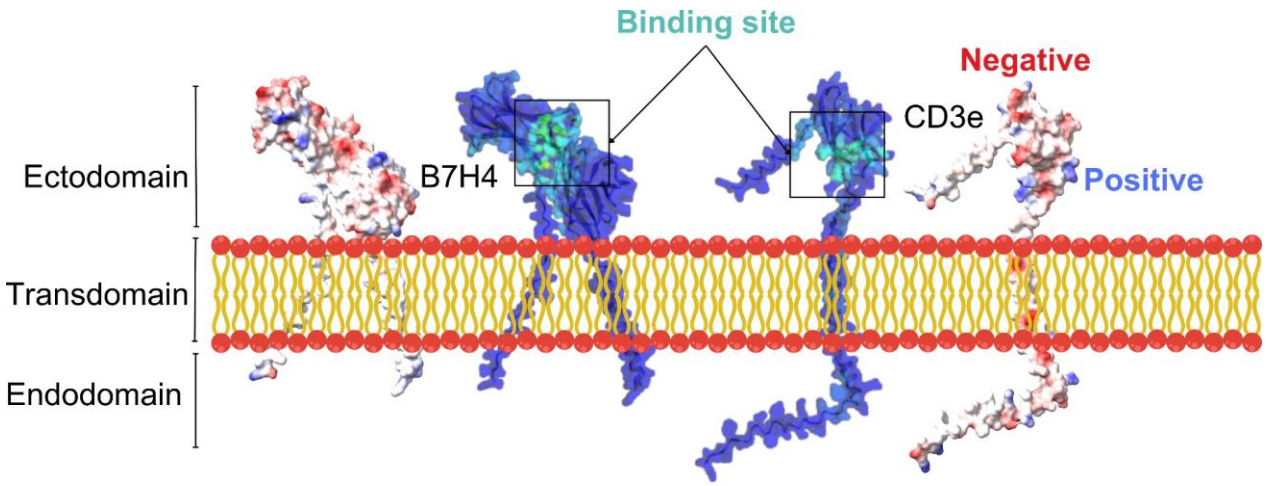

Fig. 2. The receptors present on the plasma membrane: This figure shows the B7H4 and CD3e receptors on a cell membrane. The diagram identifies the receptors' position concerning the cell, their Electrostatic surface potential (ESP), and the ideal binding site. The binding site shown in the box was predicted using the GrASP web server.

3 and 4. The antibodies were selected by visual inspection using the ChimeraX software, where the complex was chosen based on whether or not it bound to the receptor in the binding site and then if the orientation of the antibody had a coil facing away from the binding site. Based on the visual inspection, the antibodies binding to the B7H4 receptor were 1b4j, 1il1, 2hrp, 1nlb, and 1iqw, and the antibodies binding to the B7H4 receptor were 1b4j, 1nlb, 1il1, 2hrp and 1iqw. These antibodies were further selected for the following selection process. In the next step, we also performed the receptor-antibodies binding affinity calculations. Binding energy measures the smallest amount of energy required to move a particle. The binding energy is measured in kcal/mol, and the most negative binding energy number is the most robust binder. PRODIGY by Bovin Lab is a website designed to predict the binding affinity in biological complexes based on the structural properties of the protein-protein complexes. The binding energy of the antibodies is shown in Table 1, and among the five B7H4 selected antibodies, 1il1 has the highest binding affinity (-13.7 kcal/mol). On the other hand, 4A6Y had the highest binding affinity (-16.1 kcal/mol) for the CD3e.

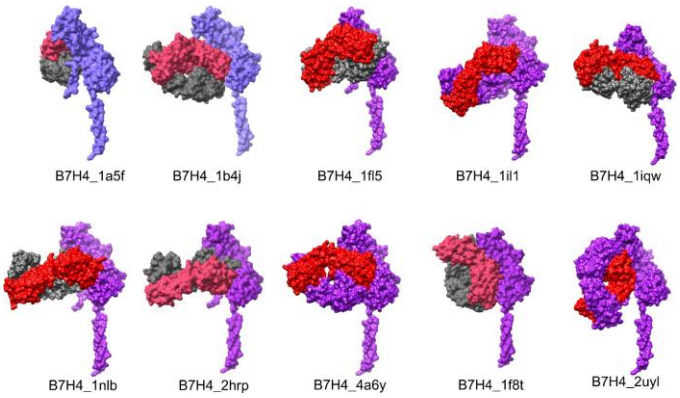

Fig. 3. B7H4-antibody interactions. From these docked structures, antibodies 1b4j, 1il1, 2hrp, 1nlb, and 1iqw bind to the binding site of the receptor surface.

3. DISCUSSION

One conventional solid tumor treatment strategy is resection, removing the tumor and some surrounding healthy tissues. Sometimes, if the entire region cannot be removed, a significant portion of the cancer tissue is removed to help reduce the subsequent treatment. Other types of cancer therapy in- clude chemotherapy, radiation therapy, and CAR T-cell therapy. Chemotherapy and radiation therapy work by damaging cancer cells so that they cannot go through the cell cycle and divide. CAR T-cell therapy is more effective for leukemia and melanoma and assists the patient's T-cells in fighting cancer.

Application and Limitations: This research can be used for targeted cancer therapy. In targeted therapy, only the specific cancer cell is destroyed, leaving the healthy or normal cells unharmed.(12) Because the B7H4 receptor is more common in solid tumors, this research applies explicitly to treatment for solid tumors.(5) In addition to treating cancer cells, the DuoBody antibody epitope can also be used to diagnose cancer when the fluorescent dye is attached to the DuoBody and binds to the cancer cell.(13) Since the antibodies and receptors are inside the body, they are surrounded by water. In future studies, we will conduct molecular dynamics simulations to ensure the antibody remains bound to the receptor surface. Since the research is computational, the next step would be to perform experimental validation. Lab experiments like ELISA (Enzyme-Linked Immunosorbent Assay) will be used to find the binding strength of the antibody.(14) In addition, due to the limited number of antibodies screened in the study, other antibodies not considered in this study could be more effective than the ones shown in our results.

Conclusion: In conclusion, the current research results suggest that antibodies 1il1 and 4a6y, targeting the B7H4 and CD3e receptors, respectively, are the most appropriate for creating a DuoBody antibody. DuoBody design holds significant potential, making the treatment better. The docking simulations helped to identify these antibodies, and the results were validated using a graph neural network. Future experimental lab experiments should be conducted to confirm the antibody specificity further.

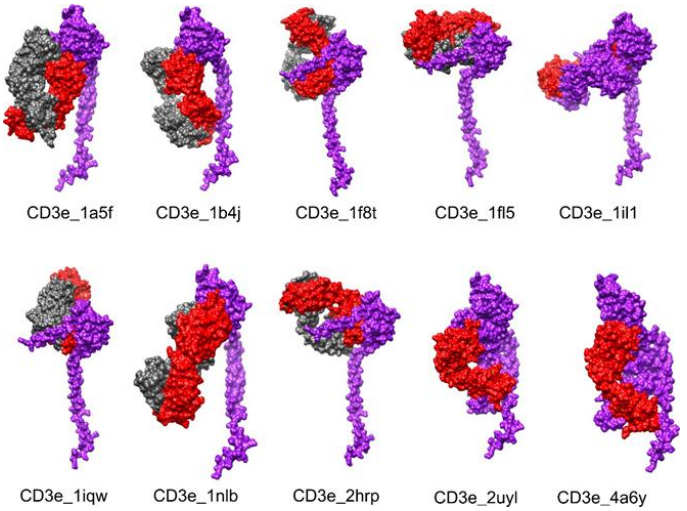

Fig. 4. CD3e-antibody interactions. Antibodies 1b4j, 1nlb, and 1a4f from these docked structures bind to the receptor surface's binding site.

4. METHOD

This research paper used the B7H4 cancer receptor and the CD3e CAR-T cell receptor. The structure of these receptors was obtained by using AlphaFold3 (15). The structures of the antibodies used in this research paper were downloaded from the RCSB Protein Data Bank.(16) Next, the binding site on the receptors was predicted using the GrASP.(17) GrASP is a graph neural network-based method to determine binding sites on protein surfaces [Figure 2]. Then, ESP (Electrostatic Surface Potential) was calculated using the ChimeraX [Figure 2].(18) Next, to understand receptor and antibody interactions, each receptor-antibody combination was modeled using the HDOCK2.0 [Figures 3 and 4].(17) The HDOCK2.0 software uses a molecular docking and template-based modeling algorithm to simulate receptor-antibody binding complexes.(17) Next, the binding energy in these complexes was computed using the PRODIGY software. (19) Figures were generated using BioRender and ChimeraX software. (18)

5. REFERENCES

1. Travis, L.B. "Therapy-associated solid tumors." Acta Oncologica, vol. 41, no. 4, 2002, pp. 323-333, doi:10.1080/028418602760169361.

2. Ramaswamy, S. et al. "A molecular signature of metastasis in primary solid tumors." Nature Genetics, vol. 33, no. 1, 2003, pp. 49-54, doi:10.1038/ng1060.

3. DeNardo, D.G. et al. "Immune cells as mediators of solid tumor metastasis." Cancer and Metastasis Reviews, vol. 27, no. 1, 2008, pp. 11-18, doi:10.1007/s10555-007-9100-0.

4. Therasse, P. et al. "New guidelines to evaluate the response to treatment in solid tumors." JNCI: Journal of the National Cancer Institute, vol. 92, no. 3, 2000, pp. 205-216, doi:10.1093/jnci/92.3.205.

5. Dawidowicz, M. et al. "B7h4 role in solid cancers: A review of the literature." Cancers (Basel), vol. 16, no. 14, 2024, doi:10.3390/cancers16142519.

6. Forthal Donald, N. "Functions of antibodies." Microbiology Spectrum, vol. 2, no. 4, 2014, pp. 10.1128/microbiolspec.aid-0019-2014, doi:10.1128/microbiolspec.aid-0019-2014.

7. Engelberts, P.J. et al. "Duobody-cd3xcd20 induces potent t-cell-mediated killing of malignant b cells in preclinical models and provides opportunities for subcutaneous dosing." eBioMedicine, vol. 52, 2020, doi:10.1016/j.ebiom.2019.102625.

8. Kontermann, R.E. et al. "Bispecific antibodies." Drug Discovery Today, vol. 20, no. 7, 2015, pp. 838-847, doi:https://doi.org/10.1016/j.drudis.2015.02.008.

9. Fan, J. et al. "Progress in molecular docking." Quantitative Biology, vol. 7, no. 2, 2019, pp. 83-89, doi:10.1007/s40484-019-0172-y.

10. Yan, Y. et al. "The hdock server for integrated protein–protein docking." Nature Protocols, vol. 15, no. 5, 2020, pp. 1829-1852, doi:10.1038/s41596-020-0312-x.

11. Koopman, L. et al. "863 in vitro and in vivo studies establish duobody®-cd3xb7h4 as a novel drug candidate for the treatment of solid cancers." Journal for ImmunoTherapy of Cancer, vol. 9, no. Suppl 2, 2021, pp. A904-A904.

12. Tsimberidou, A.-M. "Targeted therapy in cancer." Cancer Chemotherapy and Pharmacology, vol. 76, no. 6, 2015, pp. 1113-1132, doi:10.1007/s00280-015-2861-1.

13. Conroy, P.J. et al. "Antibody production, design and use for biosensor-based applications." Seminars in Cell & Developmental Biology, vol. 20, no. 1, 2009, pp. 10-26, doi:https://doi.org/10.1016/j.semcdb.2009.01.010.

14. Bobrovnik, S.A. "Determination of antibody affinity by elisa. Theory." Journal of Biochemical and Biophysical Methods, vol. 57, no. 3, 2003, pp. 213-236, doi:https://doi.org/10.1016/S0165-022X(03)00145-3.

15. Abramson, J. et al. "Accurate structure prediction of biomolecular interactions with alphafold 3." Nature, vol. 630, no. 8016, 2024, pp. 493-500, doi:10.1038/s41586-024-07487-w.

16. Rose, P.W. et al. "The rcsb protein data bank: Integrative view of protein, gene and 3d structural information." Nucleic Acids Research, vol. 45, no. D1, 2016, pp. D271-D281, doi:10.1093/nar/gkw1000.

17. Yan, Y. et al. "Hdock: A web server for protein–protein and protein–DNA/rna docking based on a hybrid strategy." Nucleic Acids Research, vol. 45, no. W1, 2017, pp. W365-W373, doi:10.1093/nar/gkx407.

18. Pettersen, E.F. et al. "Ucsf chimerax: Structure visualization for researchers, educators, and developers." Protein science, vol. 30, no. 1, 2021, pp. 70-82.

19. Vangone, A. et al. "Prodigy: A contact-based predictor of binding affinity in protein-protein complexes." Bio Protoc, vol. 7, no. 3, 2017, p. e2124, doi:10.21769/BioProtoc.2124.