Machine Learning Mediated FKBP12-Enhanced CAR T Therapy in Glioblastoma

Burden of Glioblastoma

WHY GBM IS SO HARD TO TREAT

Figure 1: The Impact and Cost of Glioblastoma(GBM) on our world(200,00 deaths/year) as well as some of the reasons why Glioblastoma is such a lethal and evasive cancer. All Images created by Finalist using Canva.

Standard Care for Newly Diagnosed Glioma

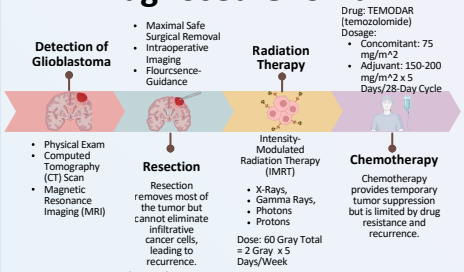

Figure 2: Standard of care for newly diagnosed glioblastoma includes a multimodal approach of Detection, Resection, Radiation therapy, and Chemotherapy. All images created by Finalist using Canva, 2025.

My Targeted Solution

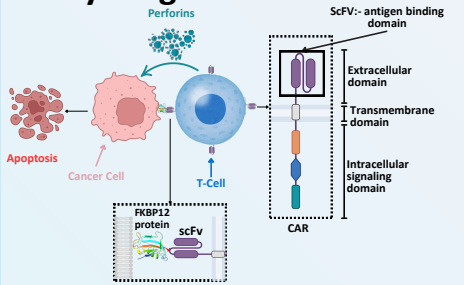

Figure 3: Schematic representation of CAR T-cell therapy targeting cancer cells. The CAR is composed of an extracellular antigen-binding domain (scFv). The scFv binds to the cancer protein FKBP12. Activation releases perforins, which initiate apoptosis in the cancer cell. All images created by Finalist using Canva, 2024.

Hypothesis

scFv antibodies targeting FKBP12 can be computationally identified and optimized using molecular docking simulations and binding energy analyses to inhibit FKBP12 function, thereby enhancing CAR T-cell therapy for glioblastoma.

Objectives

To computationally design and evaluate the binding efficiency of scFv antibodies to the FK506-Binding Protein 12 (FKBP12) for enhancing CAR T-cell therapy in the targeted treatment of glioblastoma.

Method

Protein Structure Identification: Amino acid sequence of FKBP12 obtained from UniProt (ID: P62942). Predicted 3D structure of FKBP12 using AlphaFold3.

Antibody Selection: Downloaded 3D structures of 10 antibodies from the Protein Data Bank (PDB IDs: 1A5F, 1F90, 1IGT, etc.). Selected antibodies with scFvs relevant for targeting FKBP12.

Molecular Docking Simulations: Performed docking using HDOCK to model FKBP12-scFv complexes. Visualized and analyzed docking outputs using ChimeraX.

Binding Site Prediction: Utilized ScanNet for identifying probable binding sites on FKBP12. Verified docking accuracy based on site-specific binding predictions.

Binding Energy Analysis: Evaluated binding affinity using PRODIGY to calculate ΔG values. Selected antibodies with the most negative binding energy (strongest affinity).

Hydrogen Bond Analysis: Counted hydrogen bonds between FKBP12 and scFv using in-house computational scripts.

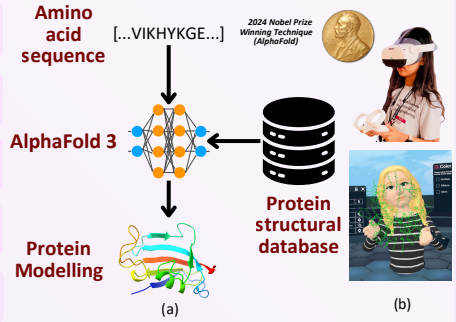

Figure 4: (a) Protein structure prediction using AlphaFold 3. All images created by finalist using Canva. (b) Image of finalist using VR goggles. All images taken by Finalist.

Results

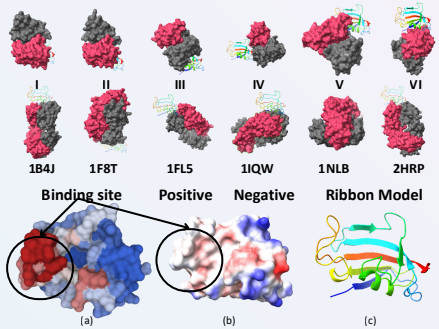

Figure 5: Surface properties of the FKBP12 protein: (a) This is the FKBP12 protein in surface form. The red part is where drugs are more likely to bind than the blue part. (b) This also shows the FKBP12 protein with the surface texture on. Red areas are more negative, blue regions are more favorable than neutral white areas, and (c) This is the ribbon form of the FKBP12 protein. It is shown for reference. Images (b,c) created by Finalist using Chimera. Image(a) created by Finalist using P2Rank, 2024.

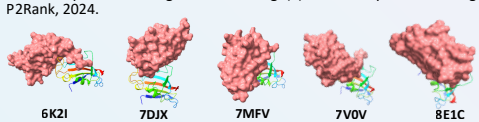

Figure 8: The above-docked structures are showing the binding of protein (rainbow) to the nanobody (pink). Image created by Finalist using Chimera.

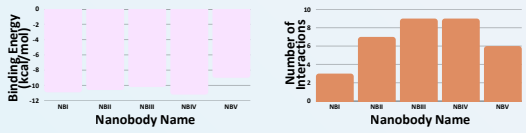

Figure 7: The binding energy and hydrogen bonds for every nanobody displayed. Higher is better as it means that the ligand is more strongly boned and has a smaller chance of falling off. Image created by finalist using PLIP and Canva, 2025.

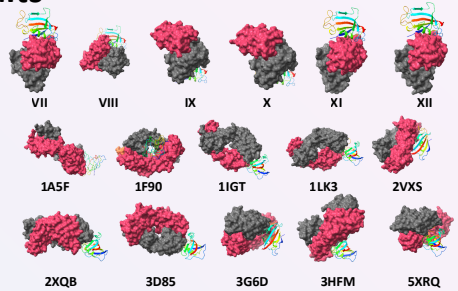

Figure 6: The above-docked structures were obtained from the HDOCK software, and ChimeraX was used to help visualize the images. Based on this analysis, only the scFvs of antibodies 1A5F, 1IGT, 1LK3, and 3D85 interact with the predicted site of the protein. Therefore, they were selected for the next analysis step. All images created by finalist using ChimeraX, 2024.

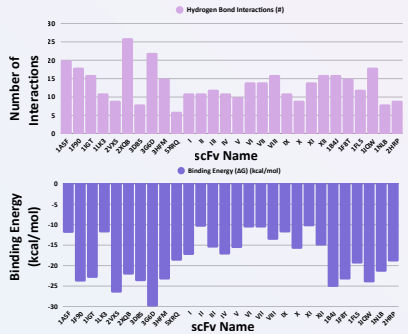

Figure 8: The binding energy and hydrogen bonds for every ligand are displayed. Higher is better as it means that the ligand is more strongly boned and has a smaller chance of falling off. Image created by finalist using PLIP and Canva, 2024.

Results Cont.

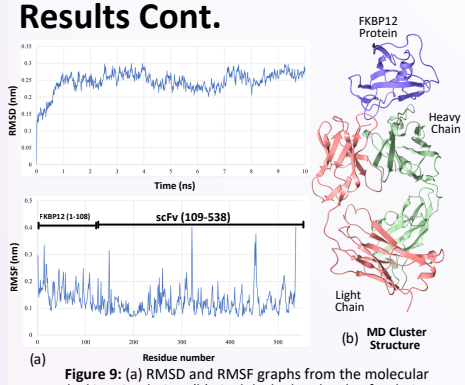

Figure 9: (a) RMSD and RMSF graphs from the molecular docking simulation. (b) Final docked molecule after being run through a GROMACS simulation. Images (a) created by Finalist using Excel, 2025. Images (b) created by Finalist using ChimeraX.

Conclusion

- 3D85 exhibited the strongest binding affinity (-30.0 kcal/mol) and highest hydrogen bonds, making it the top candidate.
- FKBP12 was validated as a promising glioblastoma therapeutic target.
- The computational pipeline was efficient for scFv screening and optimization.
- Identified scFv antibodies can inhibit FKBP12 and improve CAR T-cell therapy specificity.

Applications

- **Clinical Application:** It offers a treatment option for glioblastoma with fewer side effects compared to traditional therapies.
- **Early Diagnosis:** The antibodies can help in detecting glioblastoma early leading to faster and better treatment.
- **Surgery:** Antibody-conjugated fluorescent dye can enhance GBM tumor visualization during surgery, aiding in more precise resection.

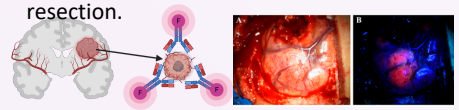

Figure 10: Early Glioblastoma detection using fluorescent dye-conjugated antibodies. Images (A, B) from: Visualization of a glioblastoma under a surgical microscope with different techniques. Note. From Effect of 5-Aminolevulinic Acid and Sodium Fluorescein on the Extent of Resection in High-Grade Gliomas and Brain Metastasis [Figure], by L. C. Ahrens, M. G. Krabbenhøft, R. W. Hansen, J. S. Sørensen, M. A. Dahl, A. Korshøj, & F. R. Hansen, 2022, Cancers, 14(3), Article 595. https://www.researchgate.net/figure/Visualization-of-a-glioblastoma-under-a-surgical-microscope-with-different-techniques_fig1_358133745 Other images created by finalist using Canva and Biorender.

Future Work

Experimental Validation: Conduct in vitro and in vivo experiments to validate the binding efficiency and therapeutic efficacy of the identified scFv antibodies targeting FKBP12.

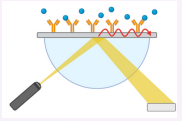

Figure 10: Surface plasmon resonance for scFV-FKBP12 binding. Image created by Finalist using BioRender, 2025

Broad Antibody Screening: Increase the antibody library to get the most effective FKBP12 inhibitor.