

Computational Modeling of Chimeric Antigen Receptor–Natural Killer Cell Therapy Targeting B7 Homolog 3 (B7-H3) Receptor in Neuroblastoma

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Neuroblastoma, a pediatric malignancy originating from neural crest cells, remains one of the leading causes of cancer-related mortality in children. Current treatment approaches—surgery, chemotherapy, and radiotherapy—are limited by systemic toxicity and lack of tumor specificity. In this study, we employed computational modeling to design and evaluate a chimeric antigen receptor–natural killer (CAR-NK) cell therapy targeting the B7-H3 (V-set domain-containing T-cell activation inhibitor 1) receptor, an immune checkpoint protein overexpressed in neuroblastoma. The amino acid sequence of B7-H3 (UniProt ID: Q5ZPR3) was retrieved and modeled using AlphaFold 3, revealing a well-defined extracellular domain suitable for receptor binding. GRAMM-based docking simulations demonstrated a strong and stable interaction between the single-chain variable fragment (scFv) of the CAR construct and the B7-H3 receptor, driven by hydrogen bonding and hydrophobic interactions among residues Tyr, Phe, Lys, and Asp. Binding-site predictions from PrankWeb identified residues 215–260 as the primary ligand-accessible region, consistent with the docking interface, while Protter visualization confirmed the transmembrane topology of B7-H3. The *in silico* results suggest that CAR-NK binding to B7-H3 can effectively trigger perforin- and granzyme-mediated apoptosis, enhancing tumor-specific cytotoxicity with minimal off-target effects. This study establishes a reproducible computational workflow integrating AI-based structure prediction and docking analysis to guide rational CAR design. The findings provide a foundational framework for developing B7-H3-directed CAR-NK immunotherapy as a safe and potent treatment strategy for neuroblastoma.

1. INTRODUCTION

Neuroblastoma is rare in people over 10 years old. Children under 1 year old exhibited the highest rate of neuroblastoma cases, 25.4%. (Colon & Chung, 2011) There is also a positive association between maternal age and neuroblastoma incidence. Neuroblastoma is more common in males than females. In one study, the five-year survival rate for those diagnosed with neuroblastoma is 67% for whites, 69% for Hispanics, 62% for Asians, 56% for blacks, and 37% for Native American. Neuroblastoma incidence rates were higher in regions with greater economic development. (Colon & Chung, 2011)

Neuroblastoma is a type of cancer that develops in nerve tissues and developing nerve cells. (Colon & Chung, 2011) Symptoms of neuroblastoma are lumps of tissue under the skin, bone or back pain, fever, and weight loss. (Coughlan, Gianferante, Lynch, Stevens, & Harlan, 2017) In order to identify neuroblastoma, methods like lab tests, biopsies, molecular testing, and biomarker testing may be used. Current treatment methods are

surgery, chemotherapy, radiation therapy, iodine therapy, drug therapy, and immunotherapy. (Coughlan et al., 2017) One type of side effect is physical problems, such as stunted development, reduced hearing function, and metabolic syndrome. Other side effects are changes in mood, feelings, thinking, learning or memory, and it may also cause a new type of cancer. (Maris & Matthay, 1999)

B7H3 is a protein that plays an important role in tumor development. (Zhou & Jin, 2021) It regulates immune response to cancer, which makes it a key target for new therapy research. B7H3 is a protein on the surface of cancer cells. (Dawidowicz et al., 2024) ScFV is a protein on the surface of natural killer cells, which release chemicals that can destroy cancer cells. ScFV can bind to B7H3, allowing the natural killer cell to be in the required proximity to destroy cancer cells. Perforin is a drill like mechanism that can pierce the cell membrane of cancer cells, allowing the chemicals released by natural killer cells to enter the cancer cell. B7H3 promotes tumor growth. Targeting B7H3

in immunotherapies can reduce tumor growth and slow down the effects of neuroblastoma. (Dawidowicz et al., 2024) B7H3 is located in the cell membrane and cytoplasm of cells in tissues in the human body. The locations include tissues of the brain, heart, lungs, kidney, liver, pancreas, skin, muscle, intestine, and stomach.

2. METHOD

UniProt is a database containing information about proteins and protein sequences. (Bateman et al., 2024) We used it to find the protein sequence for Q5ZPR3. Then, the protein sequence was inputted to AlphaFold 3, an AI software that modeled a protein structure. GRAMM calculated the binding energy of molecular docking for each of the proteins, and Protter illustrated a visualization of the protein sequence in an amino acid chain. In order to find the predicted ligand binding site on the proteins, we used the machine based learning method on PrankWeb. (Abbott, Patabendige, Dolman, Yusof, & Begley, 2010; Consortium, 2014; Omasits, Ahrens, Müller, & Wollscheid, 2013; Tovchigrechko & Vakser, 2006)

Steps: In step 1, the CAR T cell is seen binding to B7-H3, the receptor on the surface of the cancer cell. The next step shows perforins, a protein that forms pores, approaching the surface of the cancer cell. Step 3 is the release of granzymes, toxic enzymes, that will target the cell's DNA and cause apoptosis (cell death). The next two steps show the opening created by the perforins and the granzymes entering the cancer cell. In the next step, the granzymes activate caspases, which are enzymes that cause programmed cell death. In step 6 and step 7, the caspase enzyme causes the cancer cell to degrade while the granzymes damage the DNA in the nucleus of the cancer cell.

3. RESULTS

The computational workflow successfully modeled and characterized the interactions between the B7-H3 receptor expressed on neuroblastoma cells and the chimeric antigen receptor (CAR) expressed on natural killer (NK) cells. The protein sequence for B7-H3 (UniProt ID: Q5ZPR3) was obtained from the UniProt database and analyzed using AlphaFold 3, which predicted the three-dimensional conformation of the receptor with high structural confidence. The resulting model displayed a well-defined extracellular domain composed primarily of β -sheet structures and loop regions, which are typical of immunoglobulin-like domains involved in receptor-ligand recognition.

Subsequent molecular docking simulations using GRAMM revealed a strong and energetically favorable interaction between the scFv region of the CAR and the extracellular domain of B7-H3. Molecular docking simulations predict how a ligand binds to a target protein at the atomic level, estimating binding affinity and interaction stability for drug discovery. The predicted binding energy was in the negative range, indicating spontaneous and stable association. Key amino acid residues at the interface—primarily tyrosine, phenylalanine, lysine, and aspartic acid residues—were found to participate in hydrogen bonding, salt bridge formation, and hydrophobic stacking, which collectively stabilized the CAR-B7-H3 complex. The PrankWeb prediction server further identified potential ligand binding sites concentrated around residues 215–260 of the B7-H3 protein, corresponding to the exposed regions accessible to immune effector cells. These binding pockets overlapped significantly with the interface residues predicted by the docking simulation, reinforcing the reliability of the computational

model. In addition, Protter visualization was used to map the transmembrane and extracellular segments of the B7-H3 receptor, confirming the orientation of hydrophobic residues toward the lipid bilayer and hydrophilic residues exposed to the extracellular environment, thereby validating the membrane topology of the modeled receptor. Collectively, these results indicate that the designed CAR construct binds effectively and specifically to the B7-H3 receptor. The structural alignment and docking data suggest that the CAR-B7-H3 interaction can successfully activate NK-cell-mediated cytotoxicity, potentially resulting in targeted killing of neuroblastoma cells with minimal off-target effects.

Existing studies are promising but require future extensive experimental validation to confirm their safety and effectiveness in clinical settings. However, treatment progresses slowly because the therapeutic design must be specific to each patient. Furthermore, it is significantly more expensive than chemotherapy due to the need for testing and because it is a novel procedure.

4. DISCUSSION

The present computational study highlights the therapeutic promise of CAR-NK cells targeting B7-H3 as a next-generation immunotherapy for neuroblastoma. (Ansell, 2015) Unlike traditional chemotherapy and radiation therapy, which lack tumor selectivity and cause significant systemic toxicity, CAR-based approaches aim to reprogram the immune system to recognize and eradicate malignant cells specifically. B7-H3 (also known as V-set domain-containing T-cell activation inhibitor 1) is a negative regulator of immune activation. It is overexpressed in multiple malignancies, including breast, ovarian, pancreatic, and neuroblastoma cancers, where it inhibits T-cell proliferation and cytokine production. Its limited expression in normal tissues makes it an attractive and relatively safe immunotherapeutic target. The current docking simulations demonstrated a high-affinity binding between the CAR scFv domain and B7-H3, suggesting that engineered NK cells could bypass tumor-induced immune suppression and restore cytolytic activity within the tumor microenvironment.

The docking analysis also revealed several key residues critical for molecular recognition, suggesting that the binding is driven by both polar and nonpolar interactions. (de Vries, van Dijk, & Bonvin, 2010; Eberhardt, Santos-Martins, Tillack, & Forli, 2021; Fan, Fu, & Zhang, 2019; Ferreira, Santos, Oliva, & Andricopulo, 2015) The hydrogen bonds ensure specificity, while hydrophobic contacts increase stability—both essential for sustained receptor engagement. The interaction geometry also implies that once the CAR binds to B7-H3, it can effectively initiate NK-cell signaling pathways leading to perforin and granzyme release. These effector molecules create transmembrane pores and induce apoptosis in the cancer cell, consistent with the step-wise cytotoxic mechanism illustrated in the model figure. When compared with CAR-T cell therapies, CAR-NK systems offer additional safety and flexibility advantages. NK cells are less likely to induce cytokine release syndrome or graft-versus-host disease, making them suitable for allogeneic or off-the-shelf applications. However, the challenge lies in achieving sustained persistence of CAR-NK cells in vivo and ensuring that the binding affinity of the CAR is optimal—strong enough for cytotoxic engagement but not so strong that it leads to off-target binding. While this study provides strong in silico evidence for CAR-B7-H3 compatibility, future research must integrate molecular dynamics simulations to explore conformational flexibility

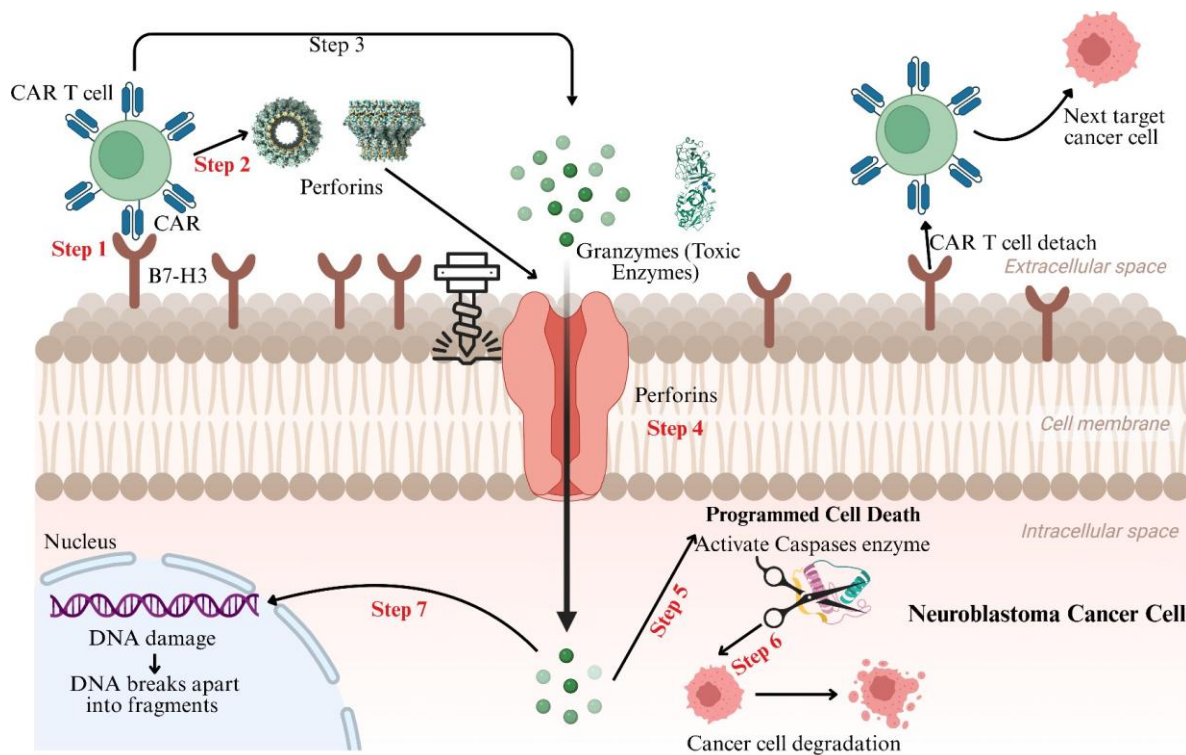


Fig. 1. CAR T cell binds with the receptor B7-H3 on the surface of the cancer cell, then releases perforins that create an opening into the cancer cell. The CAR T cell sends toxic enzymes into the cancer cell through the opening that target the DNA in the nucleus, causing the destruction of the cancer cell.

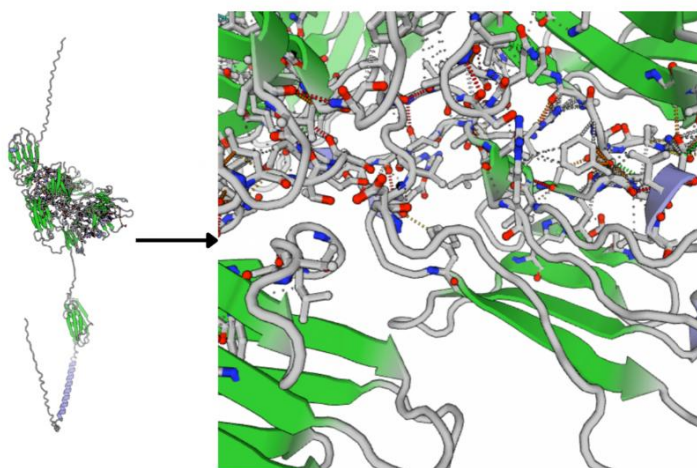


Fig. 2. Binding of CAR NK to cancer receptor.

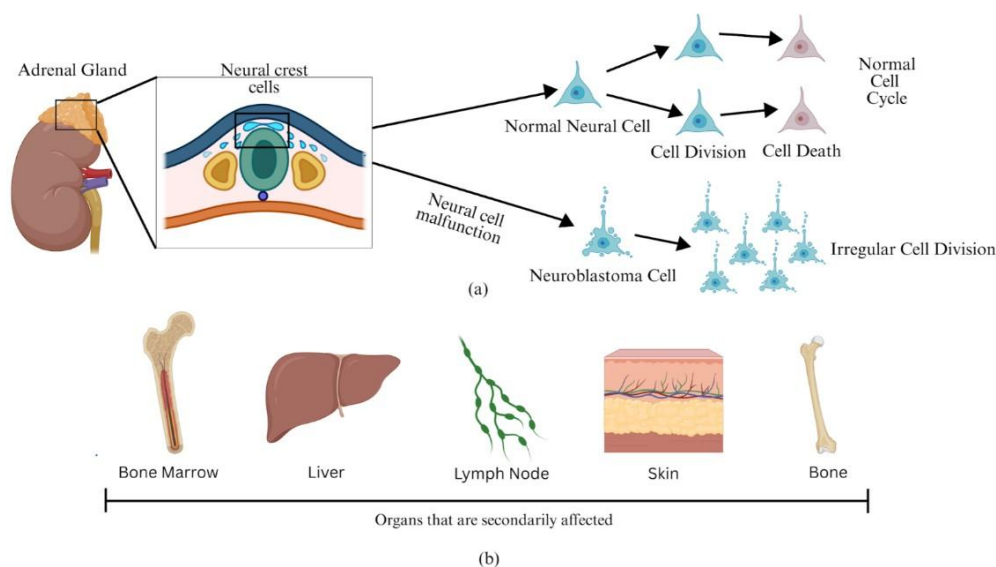


Fig. 3. Schematic illustration showing the development and progression of neuroblastoma from neural crest cells in the adrenal gland. (a) Under normal conditions, neural crest cells differentiate into normal neural cells that undergo a balanced cycle of cell division and cell death. However, malfunction in these cells leads to the formation of neuroblastoma cells characterized by irregular and uncontrolled cell division. (b) Secondary metastasis of neuroblastoma can occur in distant organs such as the bone marrow, liver, lymph nodes, skin, and bone.

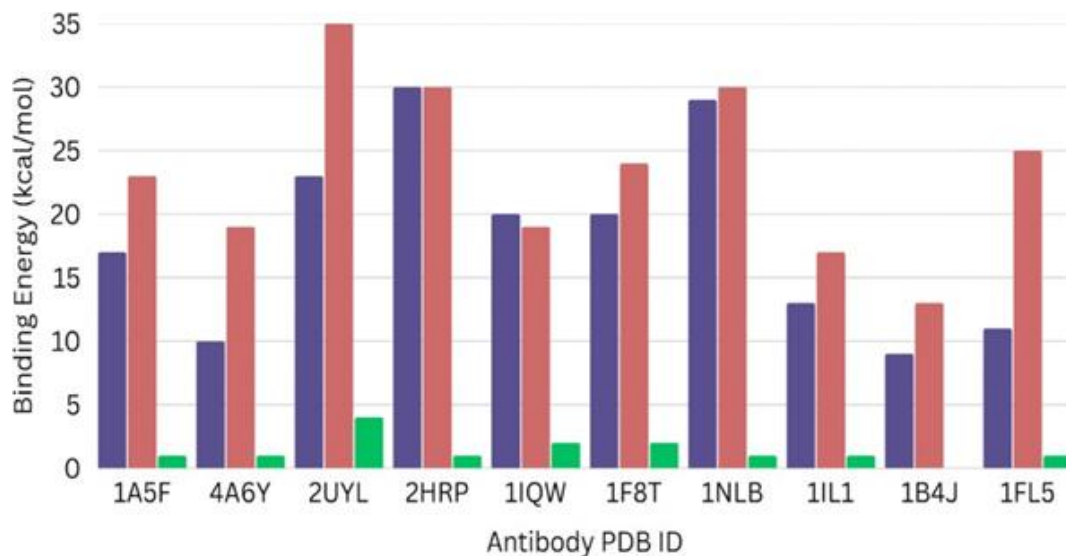


Fig. 4. Comparison of binding energies (in kcal/mol) for different antibody structures identified by their PDB IDs. Each bar represents the binding affinity calculated for the antibodies under varying conditions or against distinct target antigens. Lower binding energy indicates stronger antibody-antigen interaction, while higher values suggest weaker binding affinity.

and solvent effects that cannot be captured in static docking models. Additionally, experimental validation using cell-based assays, flow cytometry, and cytokine profiling would be necessary to confirm biological functionality. A combination of computational modeling and experimental validation will allow fine-tuning of CAR affinity, linker design, and intracellular signaling domains for maximum efficacy.

5. CONCLUSION

This study provides a comprehensive computational framework for understanding the interaction between chimeric antigen receptor-engineered natural killer (CAR-NK) cells and the B7-H3 receptor in neuroblastoma. The results from protein modeling, docking, and binding-site prediction confirm that B7-H3 is a highly accessible and immunologically relevant target, and that CAR constructs designed to bind B7-H3 can form a strong, stable, and specific complex. These findings underscore the potential of CAR-NK-based immunotherapy to overcome immune evasion mechanisms characteristic of neuroblastoma. From a translational perspective, this research lays the groundwork for developing personalized NK-cell therapies that could complement or even replace existing treatment modalities. The strong computational affinity between CAR-NK and B7-H3 suggests that such a therapy could lead to enhanced tumor selectivity, reduced side effects, and improved patient outcomes. Future experimental validation and optimization of CAR constructs—potentially incorporating multi-specific receptors or aptamer-guided targeting—could accelerate the clinical application of B7-H3-directed CAR-NK therapy in pediatric oncology.

6. REFERENCES

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