Computational Simulations of PROTAC as a BRD4 Inhibitor in Neuroblastoma

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Neuroblastoma (NB) is the most common cancer of nerve tissue in babies. The oncogene MYCN gets amplified in the disease, which can be inhibited by targeting the BRD4 protein. PROTAC, a protein degradation tool, is an emerging therapeutic strategy that targets disease-causing proteins. Two proteins are the subject of the current study project: the BRD4 protein and the E3 ubiquitin (E3) ligase protein. The BRD4 protein is crucial for controlling genes and maintaining cell viability; when it malfunctions, neuroblastoma can result. However, the activity of the n can be inhibited by the E3 protein, which also functions as a cancer suppressor. To destroy the BRD4 protein, the E3 proteins should come into proximity to each other. We hypothesize that the aptamers used in this study will bind strongly at the interface between the BRD4 and E3 ligase protein. This is made possible by a substance called PROTAC, which binds to the E3 and BRD4 proteins, causing them to come together to form the BRD4-PROTAC-E3 complex. In the present study, molecular docking was employed to elucidate the BRD4-PROTAC-E3 interactions, and based on this understanding, we designed 10 new PROTACs and evaluated their medicinal properties. Based on our analysis, Aptamer II formed strong interactions with both the proteins. The discovery of new PROTACs will facilitate the computer-aided design of robust, cost-effective, and minimally side-effecting PROTACs for cancer treatment.

1. INTRODUCTION

Neuroblastoma is a very rare cancer which develops from immature nerve cells, called neuroblasts [1]. Instead of becoming functional cells, their uncontrollable growth leads to the formation of a tumor [1]. Neuroblastoma often originates in neuroblasts of the adrenal gland tissue, an endocrine gland situated on top of the kidneys [2]. Hormones produced by the adrenal gland help regulate blood pressure, metabolism, and the body's response to stress (Miller, O'Callaghan, & Experimental, 2002). Neuroblastoma can also originate in other body parts that contain clusters of nerve cells and may metastasize to other parts of the body [3]. Neuroblastomas primarily affect babies and adolescents, usually under the age of five and rarely older than ten, because they have the most nerve cells that are still developing [4]. Symptoms of a neuroblastoma include a lump that can be seen or felt, swelling, and more, based on the location of the tumor [5]. Neuroblastoma is diagnosed through physical exams, blood and urine tests, imaging, and tissue and bone marrow biopsies [6]. There are four stages of neuroblastoma: the lowest risk stage, L1, where tumors are in one part of the body, don't affect vital structures, and haven't spread; L2, where the tumor is still in one body compartment, but vital structures are involved and cancer cells have spread to regional structures like lymph nodes; M, when the cancer has become metastatic; and the "special" MS category, which is when the neuroblastoma has extended to the bone marrow, liver, or skin in children younger than eighteen months, and this stage is considered low risk [7]. Neuroblastoma can be treated through surgery, chemotherapy, immunotherapy, radiation therapy, and bone marrow transplants [8].

PROTAC, which stands for Proteolysis Targeting Chimera, induces the ubiquitination- and degradation-mediated removal of target proteins [9]. It has two active domains connected by a linker, and these domains interact with E3 Ligase, specifically in the case of neuroblastoma, the Bromodomain and Extra-Terminal (BET) proteins, which are crucial for cell functioning and gene regulation [10]. Its malfunctioning can lead to the uncontrollable replication of cells, which can mutate and result in neuroblastoma. The ubiquitin on the E3 Ligase transfers to the BET and marks it for destruction [11].

Less than 50% of patients with invasive neuroblastoma who have MYCN gene overexpression survive, and recurrence rates are incredibly high. (Campbell et al., 2024). Thus, focusing on the MYCN protein may be crucial for treating cancer (Campbell et al., 2024). However, due to the complex structure of MYCN, focusing on manipulating the proteins that interact with MYCN

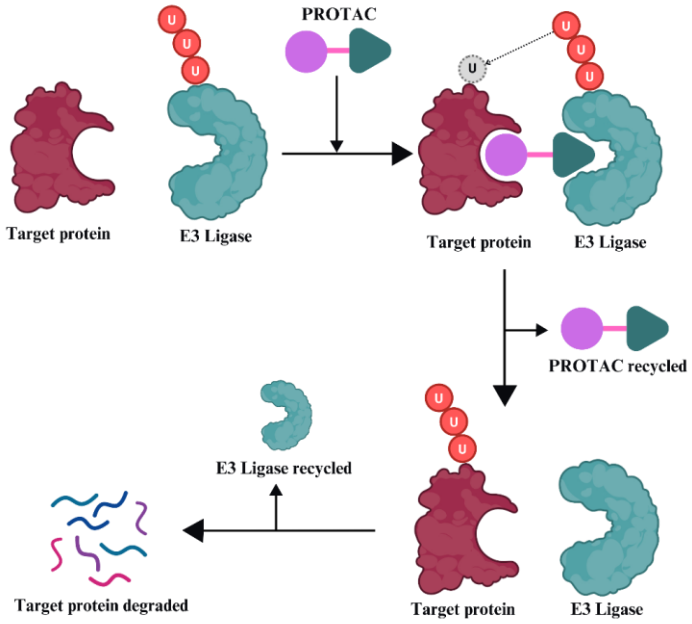

Fig. 1. Mode of operation of PROTACs. The PROTAC has two active domains connected by a linker. The BRD4 target protein is bound by one active domain, while the E3 ubiquitin ligase is bound by the other. The linker enables the E3 ubiquitin ligase and BRD4 target protein to come into close proximity, allowing the E3 ligase to transfer its ubiquitin molecules to the target protein. The proteasome identifies and breaks down the ubiquitinated target protein.

to indirectly regulate it can be an alternative strategy (Campbell et al., 2024). BRD4 is one of the proteins that controls MYCN activation by binding to a specific site on DNA, and inhibiting BRD4 can directly affect MYCN activation, thereby mitigating neuroblastoma [12]. PROTACs bring the cancer-suppressing E3 ubiquitin ligase in close proximity to the BRD4 protein, allowing for the degradation of BRD4 [13]. THE E3 ligase ubiquitin inhibits BRD4 protein activity [13]. Recently, Jia. et al. have developed PROTACs against neuroblastoma that induce the degradation of BRD4 proteins, which are ultimately targeted by the E3 ligase protein [14]. We hypothesize that the aptamers used in this study will interact strongly at the interface between the BRD4 and E3 ligase protein. The current investigation explores the identification of inhibitors that can bind to both proteins (BRD4 and E3 ligase) and could help in the ubiquitination of the BED4 leading to its proteasomal degradation. In particular, we have investigated the interactions of these molecules through molecular docking simulations. PROTACs to the BRD4-L3 ligase protein. Moreover, mutations were performed in the PROTACs structure to enhance their binding affinity. This research will aid in the design of PROTACs that exerts potent anticancer effects against neuroblastoma.

2. METHOD

The initial input file contains three components; (a) E3 ligase, (b) BRD4 protein, and (c) PROTAC. The E3-PROTAC-BRD4 protein was from Protein Data Bank (6BOY), a free database for large biological molecules (Nowak et al., 2018). From this complex the PROTAC molecules were first retrieved and were mutated using the following steps: First, the PROTAC was converted to the mol2 file format. The PROTAC.mol2 file was then mutated to ten different PROTAC variants in SMILES format. The CReM (Chemically Reasonable Mutations) is a computational tool to perform chemical mutations of molecules to obtain 10 mutated PROTACs [15]. In the next step, the mutated PROTACs were converted to PDB format to perform molecular docking simulations. The molecular docking was performed between E3-BRD4 complex and mutant PROTACs using the HDOCK2.4 software to obtain the E3-PROTAC-BRD4 complexes (Yan, Zhang, Zhou, Li, & Huang, 2017). Molecular docking simulations gave 10 different E3-PROTAC-BRD4 complexes which were later analyzed by using the PLIP website server to obtain the interactions formed between protein-PROTAC complexes [16]. The protein's binding location were found by GrASP and P2Rank website server (Krivák & Hoksza, 2018). The obtained complexes were further analyzed using ChimeraX (Pettersen et al., 2021) and PyMol (Yuan, Chan, & Hu, 2017) software.

3. RESULTS

We studied the PROTACs that can bind to both BRD4 and E3 ligase protein. The present study investigated how PROTACs inhibit the BRD4 protein and can aid in treatment of neuroblastoma. The present study was grounded on the recent discoveries of Jia et al., who found that BRD4 protein inhibitors manifest anti-neuroblastoma activity. Their research utilizing mice showed that the BRD4 protein suppression by PROTAC was dose-dependent, leading to apoptosis and cell-cycle arrest [14]. In order to investigate protein-PROTAC interactions, our re-

search employed in silico methodologies like molecular docking simulations. In addition, we also generated the PROTAC library by performing mutations in PROTAC. Ultimately, the most effective PROTAC has been identified based on the PROTAC interactions formed with both proteins. The current work comprises 10 PROTACs and for the sake of simplicity they are designated as PROTAC I, II, III, IV, V, VI, VII, VIII, IX, and X.

Using the Graph Attention Site Prediction (GrASP) website server, we applied graph neural networks (GNN) to verify the fact that the PROTAC attaching location is the druggable site [17]. Graph Neural Networks (GNNs) are able to record the connections and interactions between nodes in a graph, in contrast to typical neural networks that process organized data such as scenes or images. The molecules are shown as graphs with bonds acting as edges and atoms as nodes. The probable drug binding site was then displayed, and the most important areas of the graph were highlighted by the attention mechanism. In Figure 2a, the druggable region is displayed. This meant that the druggable site, which matched the EGF binding region in color, was displayed as yellow and green. This was further verified by predicting the druggable site (Figure 2b) employing P2Rank, a machine learning technology. The capacity to precisely target and bind medications to particular locations on proteins was symbolized by the term "druggable site." The process of creating therapies that can attach to these biomolecules requires the identification of druggable sites.

PROTAC mutation: To obtain different mutations in PROTACs, CReM also called the Chemically Reasonable Mutations framework was utilized [15]. CReM mutations are done by fragmenting the molecule into smaller subunits and are stored in a database. Next the mutations are performed by replacing fragments with other fragments from the database. During this step the valency, aromaticity, and functional groups are considered to ensure that the resulting molecules are chemically reasonable. Finally, multiple new structures are generated through iterative mutations. Based on the CReM web server, we obtained 10 PRO- TACs in a SMILES format [15]. SMILES are a chemical representation method that allows computers to process chemical compounds.

The obtained PROTACs were docked at the proteins' active site, and the PROTAC-protein interactions were calculated via the web server PLIP [16]. According to PLIP study: The con- trol formed 14 hydrophobic bonds, four hydrogen bonds, and one π-cation bond. PROTAC I formed 10 hydrophobic bond, 2 hydrogen bonds, 3 π-cation bond, a halogen bond, and a salt bridge. PROTAC II formed 12 hydrophobic bond, 3 hydrogen bonds, and 1 π-cation bond. PROTAC III formed 11 hydropho- bic bond, 3 hydrogen bonds, and a π-cation bonds. PROTAC IV formed 9 hydrophobic bond, 1 hydrogen bond, and 4 π-cation bonds. PROTAC V formed 5 hydrophobic binds, 2 hydrogen bonds, and 3 π-cation bonds. PROTAC VI formed 9 hydropho- bic bond, 2 hydrogen bonds, a π-cation bond, and a salt bridge. PROTAC VII formed 9 hydrophobic bond, 4 hydrogen bonds, and 3 π-cation bonds. PROTAC VIII formed 10 hydrophobic bond, 1 hydrogen bond, and 2 π-cation bonds. PROTAC IX formed 6 hydrophobic bond, 4 hydrogen bonds, and 2 π-cation bonds. PROTAC X formed 10 hydrophobic bond and a hydrogen bond.

4. DISCUSSION

In this study, we employed computational methods tools to find PROTACs against Neuroblastoma [1]. It is among the most prevalent solid tumors of the sympathetic nervous system in children [7]. Recurrence is extremely common and, regrettably, less than 50% of patients with invasive neuroblastoma who have MYCN gene overexpression survive [3]. Therefore, targeting MYCN protein could be crucial to the treatment of cancer [18]. However, due to the complex structure of MYCN focusing on manipulating the proteins that interact with MYCN to indirectly regulate it can be an alternative strategy [18]. BRD4 is one of the proteins that controls the MYCN activation by binding to a specific site of DNA and inhibiting BRD4 can directly affect the MYCN activation mitigating neuroblastoma [19]. PROTAC were designed against cancer inducing BRD4 proteins which are eventually degraded by the E3 ligase protein, a cancer suppressor, is essential for destroying BRD4 proteins by bringing them in proximity via PROTAC. Computational tools used to understand E3-PROTAC-E3 interactions and design 10 novel PROTACs. PROTAC II showed the most favorable binding site. Findings suggest potential for developing potent, cost-effective PROTACs against cancer, aiding in reducing the disease's worldwide impact. The accuracy of molecular docking mainly depends on the initial structure and algorithm employed [20–22]. Consequently, molecular dynamics simulations are necessary to further verify the docking findings.

The Figure 4 illustrates the projected global market growth for pediatric neuroblastoma treatment from 2024 to 2029. Beginning at $1.85 billion in 2024, the market is expected to steadily rise each year, reaching $2.87 billion by 2029. This consistent upward trend reflects a compound annual growth rate (CAGR) of 9.1%, indicating strong and sustained investment in the development of treatments for this rare pediatric cancer. The increase in market size may be attributed to advances in targeted therapies, immunotherapy, improved diagnostic techniques, and growing awareness of childhood cancers. The steady rise in funding, clinical research, and innovation in drug development further supports this growth. Overall, the graph highlights a promising future for the pediatric neuroblastoma treatment market, signaling expanding opportunities for healthcare providers, biotech firms, and pharmaceutical companies to improve outcomes for affected children worldwide.

Summary of standard and experimental treatment methods for neuroblastoma, categorized by type, mechanism of action, and clinical usage.

BRD4 is also linked to Alzheimer's disease, in which BRD4 inhibition increases brain cognitive ability. In addition, blocking this protein also prevents the neuro dysfunction caused by brain stroke. dBET57 disrupted BRD4 function, leading to the downregulation of critical super-enhancer-associated genes, which resulted in inhibited cancer cell growth and induced apoptosis. Recently, using experimental methods, the possibility of using dBET57 as a medicinal agent for neuroblastoma has been discovered [23, 24]. Additionally, various clinical trials are being conducted that target the BRD4 protein for the treatment of hematological and solid cancers [25–29]. This preclinical research has also suggested that chemotherapeutic drugs can be used in combination with BET inhibitors. A few kinds of chemotherapeutic medications, such as proteasome inhibitors, tyrosine kinase inhibitors, CDK inhibitors, and immunotherapies, have been found in recent preclinical research to be useful in conjunction with BET inhibitors to improve therapeutic results and circumvent BET inhibitor resistance. Therefore, it is going to be crucial also to design biomarkers to compute the efficacy of these drug blends. Moreover, the identified BET inhibitors have a short half-life and require further chemical modification, as well as detailed pharmacokinetic and pharmacodynamic evaluation of these compounds.

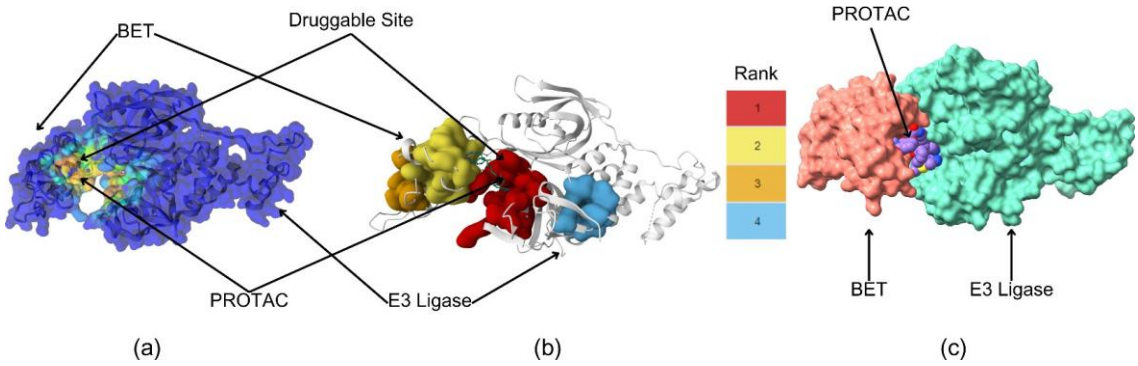

Fig. 2. Druggable site on BRD4-E3 Ligase complex.(a) The GrASP website host was used to forecast the druggable site, which is displayed in yellow and green; (b) The BINDING spot was further confirmed by a machine learning tool called P2Rank. In this image the red and yellow region are the interacting location; and (c) shown for reference. In this image the BRD4 protein is in salmon and E3 Ligase is in turquoise.

Table 1. Summary of standard and experimental treatment methods for neuroblastoma, categorized by type, mechanism of action, and clinical usage.

Treatment Method	Type	Mechanism	Typical Use / Indication
Surgery	Local Therapy	Removal of primary tumor	Early-stage neuroblastoma; tumor resection
Chemotherapy	Systemic Therapy	Cytotoxic drugs that kill fast-dividing cancer cells	Intermediate/high-risk neuroblastoma; pre/post-surgery
Radiation Therapy	Local Therapy	High-energy rays to kill or shrink tumors	When tumor is unresectable or post-surgery to kill remnants
Autologous Stem Cell Transplant	High-dose Therapy	Replaces bone marrow after intensive chemotherapy	High-risk neuroblastoma following induction chemotherapy
Immunotherapy (e.g., anti-GD2)	Targeted Therapy	Monoclonal antibodies target neuroblastoma cells expressing GD2 antigen Promotes	High-risk neuroblastoma; often used after stem cell transplant
Retinoid Therapy (e.g., 13-cis-retinoic acid)	Differentiation Therapy	maturation of neuroblastoma cells to non-cancerous forms	Maintenance therapy after initial treatments for high-risk
MIBG Therapy (I-131 MIBG)	Targeted Radiotherapy	Radioactive iodine linked to MIBG taken up by neuroblastoma cells	Relapsed/refractory neuroblastoma
CAR T-cell Therapy (experimental)	Immunotherapy	Genetically engineered T-cells target tumor-specific antigens	Clinical trials for relapsed or refractory neuroblastoma
Clinical Trials	Experimental	Novel therapies (gene therapy, new biologics, etc.)	For treatment-resistant or relapsed cases
Supportive Care	Symptom Management	Pain relief, nutritional support, infection control	Throughout treatment to improve quality of life

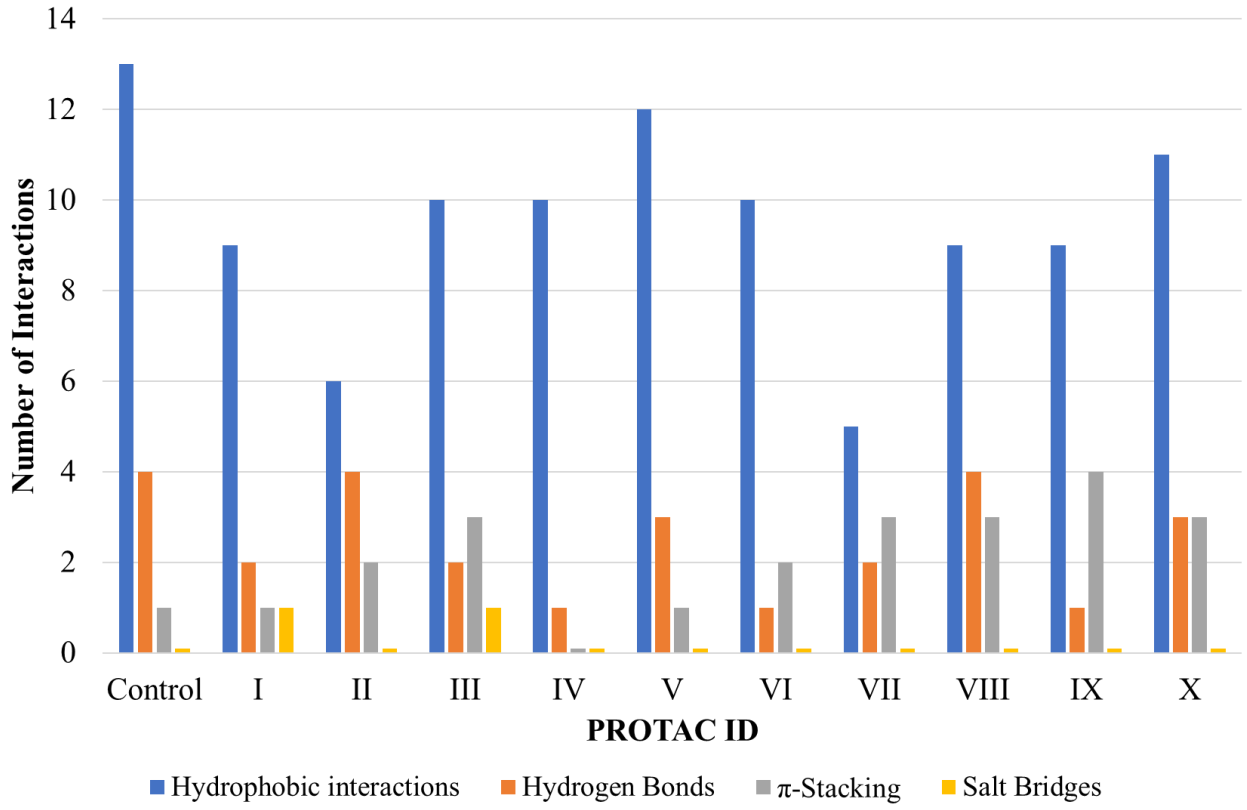

Fig. 3. The number of interactions formed in the complex. Hydrophobic interactions were the most prevalent in all the protein-PROTAC complexes.

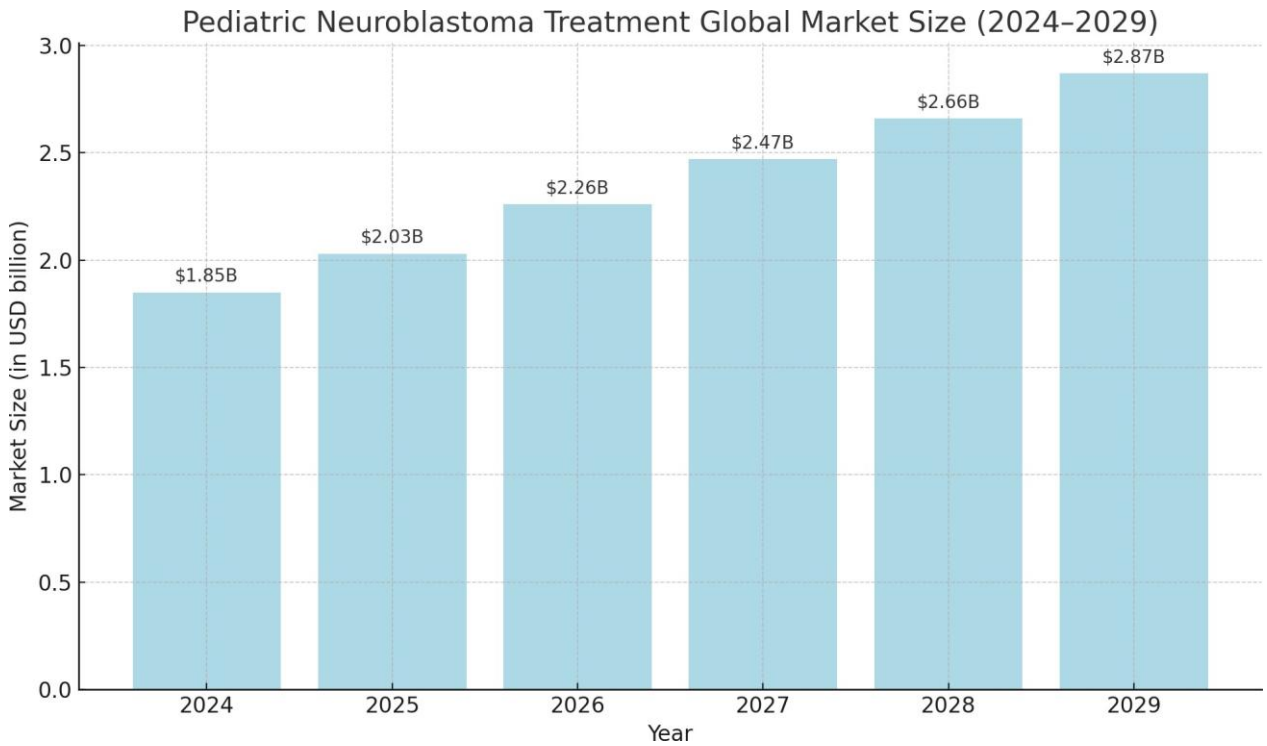

Fig. 4. Projected growth of the global pediatric neuroblastoma treatment market from 2024 to 2029, showing a steady increase from $1.85 billion in 2024 to $2.87 billion in 2029, with a compound annual growth rate (CAGR) of 9.1%.

In order to treat neuroblastoma, the study used computational methods to create and assess novel PROTACs that target BRD4, a protein involved in gene regulation and cell functioning. Using chemical modifications to create a PROTAC library and molecular docking simulations, the study found that PROTAC II was the most efficient at binding to E3 ligase and BRD4 proteins. This interaction stimulates BRD4's ubiquitination and subsequent destruction, which stops neuroblastoma cells from proliferating. The results imply that these engineered PROTACs have the potential to be developed into effective, affordable, and less side-effect-prone therapeutic approaches for the treatment of neuroblastoma. To validate these computational results, more validations from experiments and molecular dynamics simulations are required.

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