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Antibacterial Toxin-Derived Immunotoxins: Innovative Constructs for Targeted Breast Cancer Treatment

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Abstract:

Cancer remains one of humanity's leading causes of both illness and death globally. In women worldwide, breast cancer remains the most widespread malignant condition. The new possibilities for direct treatment offered by the advances made thereby were the subject of the recent study undertaken as it sought to unravel tumorigenesis through genetics and molecular appreciation of cancer. Specifically, this research centers on devising and testing immunotoxins as anti-bacterial toxin-based constructs to treat breast cancer. These immunotoxins can kill cancer cells selectively while leaving normal tissues unharmed as they bind only to cancer cell antigens by using both the specificity of antibodies and bacterial toxins' cytotoxicity power. We assessed immunotoxins' binding affinities to their respective antigens based on computational dockings like HADDOCK explaining encouraging results characterized by good docking scores accompanied by low RMSDs—also, dual targeting approaches combined with structure-based. Challenges such as immunogenicity and non-specific toxicity have been tackled by developing humanized antibodies and novel targeting moieties. Our findings suggest that optimized immunotoxins have great potential to enhance therapeutic window as well as efficacy in cancer treatments.

INTRODUCTION

Cancer grows uncontrollably and spreads sometimes in the body too. However, it is also caused by genetic mutations and other factors. Given the recent research findings, this integration is intended to offer a lucid view of what is currently known about this disease as well as its therapies, especially breast cancer (1).

Genetic Basis of Cancer

In essence, cancer stems from errors in genes, which must occur in multiples before a tumor may develop and spread in the body (2-4). Ground-breaking discoveries have been made in the areas of cancer genetics, which have unveiled principal transformations and routes connected to several types of cancer, thus opening doors to fresh treatment options (4).

Physical Traits of Tumors

Tumors possess specific physical characteristics including heightened solid stress, greater interstitial fluid pressure, rigidity, and changes in microstructure that lead to tumor progression and resistance to treatment.

Breast Cancer Specifics

The most frequent cancer in women globally is cancer affecting their breasts which, if discovered before it advances further are curable, but sometimes remain incurable when detected at later stages (5-7). Treatment strategies depend heavily on molecular subtypes. The molecular subtypes include the hormone receptor status and HER2 expression. Involving is a combination of surgery, radiation, endocrinal therapy as well as chemotherapeutics targeted at controlling cancer cells within breast tissues (7). The standard strategy has changed for treating breast cancer, particularly HER2-positive and triple-negative breast cancer using first-line treatment (5, 7).

Cancer Incidence and Mortality Trends

The rate of cancer occurrence and death is different depending on what type of cancer it is and some basic demographic characteristics. Breast cancer incidence is still escalating, whereas vaccination against HPVhas led to a drastic reduction in cervical cancer incidences (8, 9). Advances in treatment and early detection of cancer have led to reductions in its mortality despite

increasing incidences of some cancer types (8).

Tumor Microenvironment and Immunology

Cancer progression and response to treatment are greatly influenced by the tumor microenvironment, which comprises immune cells and physical conditions (8, 10). Incorporating immune parameters into clinical stratification schemes can improve prognostic and predictive information, guiding better clinical decisions (11). The cancer research field has made some noticeable advances in comprehending the genetic and physical bases of this ailment. Such discoveries result in better and more precise treatment methods being developed. Breast cancer for example is the best representation of this complexity seen in cancers where different types are responsive to different therapeutic agents at specific stages of one's life. This means that when you look at someone's blood samples under those circumstances they have molecular subtypes which have no resemblance with each other just like any other disease out there would be different within them. Advances made in immunology enable us to appreciate the contribution made by the tumor microenvironment towards personalizing patient-centered oncology.

Even with these new possibilities, we must address some serious issues in the future. For example, there's an increasing number of breast cancer cases that need specific treatment options. Antibiotics that release toxic substances ii immunotoxins are a new idea for fighting breast cancer. The antibody that identifies cancer cells combined with toxic substances from bacteria is the basis for immunotoxins meant to destroy these malignant cells without affecting other parts of the body.

Targeted Therapy with Immunotoxins

Immunotoxins are designed to target cancer cells by binding to certain antigens or receptors on the surface of these cells that have been overexpressed, bringing along a powerful toxin inside them to kill in destiny (12, 13). These constructs commonly contain toxins from bacteria, such as Pseudomonas exotoxin (PE) and diphtheria toxin (DT) (14, 15).

Clinical Efficacy and Trials

Some immunotoxins have indicated hopeful outcomes in clinical tests as they suggest potent anti-cancer impacts with chances of curing cancers that do not respond to standard therapies (13, 15-17). Denileukin diftitox was the first FDA-approved immunotoxin, followed by tagraxofusp and moxetumomab pasudotox. These drugs exploit bacterial toxins to kill cells (17).

Dual Targeting Strategies

When studying breast cancer models, the combination of HER2 and EpCAM toxins that target both primary tumors and metastases would be more effective, according to dual targeting strategies.

Challenges and Improvements

Immunogenicity and non-specific toxicity are major challenges that cause serious problems, resulting in conditions such as vascular leak syndrome(18). To minimize immunogenicity different approaches are used such as using humanized or less immunogenic forms of bacterial toxins and creating new homing devices (15, 18).

Mechanisms of Action

Some Immunotoxins act by joining when cancer cell receptors, are internalized plus eventually deliver poison in cytosol that will inhibit protein synthesis leading to the death of cells. Other modifications exist in triterpenoid saponins or the like which can lead to valuable increases in tumorcidal potentiality of immunotoxins (19). Specific targeted toxins derived from antibacterial materials could be a more effective strategy in treating breast cancer. Combining the specificity of antibodies with cytotoxicity from bacterial-derived toxins ensures complete eradication of cancerous cells with minimal harm on normal ones. Despite obstacles such as immune response stimulation or high levels of unspecific toxicity, improved outcomes are anticipated as research continues in this area including dual targeting approaches to enhance therapeutic outcomes without harming healthy tissues (1).

Evaluation of Antigen and Immunotoxin Docking Capability

Docks between antigens and immunotoxins are an area of research that is very important and has great implications for developing drugs, especially in terms of cancer therapy and immune system control processes. The focus of this review is to provide an overview based on diverse computational as well as experimental investigations about what current limits exist about antigen or immunotoxin docking procedures (20).

Antigen Docking

Forecasting the interaction of such immunity agents through simulation (antigen docking) can help predict immune response and aid in the production of healing antibodies. There have been various computer-based methods aimed at improving the correctness as well as speed of their projections.

Information-Driven Docking

For example, HADDOCK, a software for docking, has been combined with information regarding the complementarity-determining regions and binding

epitopes, and has been established to perform far better than other software suites like ClusPro, LightDock, and ZDOCK, both in terms of success rate and model quality.

Surface Complementarity

An innovation in positive superposition, which uses rough energy of the steric interactions in the interface region as a scoring function, has been used with antibody-antigen docking. The protocol is shown to be sufficiently accurate to recover near-native structures and recognize feasible docking orientations with root-mean-square deviations (r.m.s.d.) from 1.9 to 4.8 A (21).

Epitope Mapping

PIPER-Map, a protocol that combines template-based modeling and docking, has improved epitope prediction accuracy, showing that starting from antibody sequence alone can yield results comparable to those starting from unbound antibody structures.

Soft Docking Algorithms

Soft-docking techniques demonstrating potential in forecasting binding sites of antibody-antigen interactions, have been developed for camelid VHH antibody variable domains using side-chain flexibility and combined filtering approaches (22).

Immunotoxin Docking

Immunotoxins are molecules constructed by two persons that bring together the specificity shown by the monoclonal antibodies and toxicity of poisons; their action is pointed at killing cancer-preceding antigens. The therapeutic efficiency of such immunotoxins is pegged on how they attach and get into the cells.

Trimeric Immunotoxins

An innovative form of the trimeric immunotoxin, which aims at CEA, managed to kill more cells in xenografts of human colorectal cancer than its monomeric analog did, implying that immunotoxin design should be carefully considered as regards docking effects (23).

Internalization Efficiency

A recent study investigated how immune toxins against CD19 and CD22 are internalized differently. This study shows that the toxins targeting CD22 are internalized quickly, leading to more killing effects on a cell, thus making it more suitable for treatment than other diseases whose immune toxins do not work well inside. We can conclude based on the data presented here that different degrees of intercalation lead to various levels of toxicity for cancer cells using CD_19-based antigen ligand conjugated to recombinant diphtheria toxin (some of which kill rapidly while some don't kill any target cells at all) which implies that there is significant difference in activity between the conjugate single chain fragments with recombinant diphtheria toxin based on their rate and capacity for internalization (24). In antigen evaluation, it is generally accepted that there have been significant improvements in computational methods for information-driven docking, surface complementarity, and soft-docking algorithms. These methods greatly advanced the modeling of antibody-antigen interactions and supported the design of the most effective immunotoxins. Moreover, future research should be aimed at advancing these techniques and exploring their development in a therapeutic sense.

1.Modeling Antibody-Antigen Complexes by Information-Driven Docking (20).
2.New policy to mimic protein interaction based on surface complementarity application on antibody-antigen docking (21).
3.Epitopes of antibodies could be mapped out using homology modeling and docking (25).
4.Antibody-antigen complex structure can be predicted using an elastic docking algorithm (22).
5.Antitumor activity of human colorectal cancer xenografts is significantly enhanced by a novel CEA-Targeted Trimeric Immunotoxin that targets CEA (23).
6.Differential cellular internalization of anti-CD19, CD34 and -CD22 immunotoxins results in different cytotoxic activity

Materials and methods

Effective therapies in the sphere of immunology and cancer research typically hinge on accurate targeting and transportation of therapeutic agents to specific cell targets. For example, the cell-surface proteoglycan CD44 is one such target that has been highly investigated because of its involvement during cancer progression and metastasis However, predicting an antigen and its receptor is a problem characterized by borderline energy levels when analyzing the binding affinities of immunotoxins including energy computations—we also consider the energy levels instead of just focusing on these computations. (Figure 1).

The evaluation of the binding affinity of an immunotoxin to its antigen was very important to determine its effectiveness and specificity (Table 1).

Evaluation of binding affinity of immunotoxin to control antigens

Bispecific Antibody-Toxin Conjugates for T Cell Subset Depletion A bispecific antibody recognizing CD34 and CD22 was created to find CD22+CD34 bright T cells. It showed that binding bidirectionally to these both antigens enhanced toxins intracellular entrance and release, which resulted in the killing of just a few cells. Two studies reviewed in this paper provide

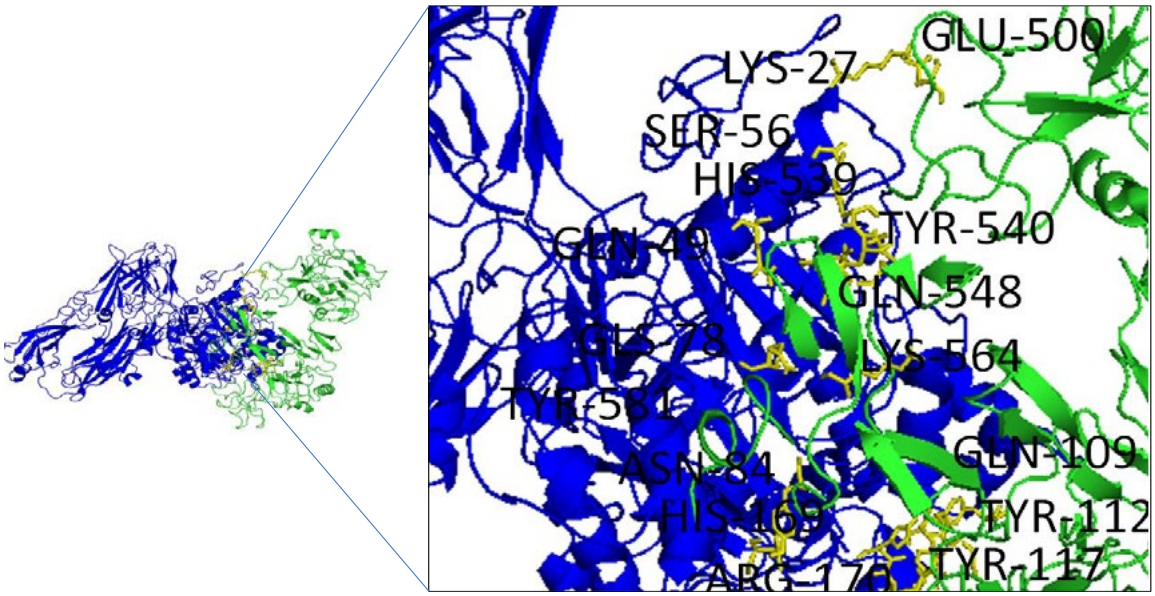

Fig1. Evaluation of binding affinity of immunotoxin and antigen

Table 1. Immunotoxin and antigen binding affinity evaluation table

HADDOCK Score	-16,3
RMSD	2,2
Z-Score	-2,4

useful insights into the development and optimization of immunotoxins for therapeutic purposes, thus revealing the importance of dual binding in boosting immunotoxin internalization and cytotoxicity; it is still unclear whether these cells use intracellular pathways to induce receptor-mediated endocytosis. Their binding abilities do not just determine the interaction between immunotoxins and normal antigens; rather, it is a composite process involving specificity, internalization, and serum half-life (Figure 2,3)2

The comparison of the binding affinity of antigens with immunotoxins reveals significant insights into the relationship between antigen binding and immunotoxin efficacy (Figure 4).

RESULTS

To determine their effectiveness and specificity in targeting cancer cells, we must assess the ability of different immunotoxins (IT) to bind to various antigens. Here, we employed HADDOCK (High Ambiguity Driven protein-protein DOCKing) software, which also happens to be very common in the docking field to study how these two agents interact with each other.

The HADDOCK score indicates the predicted quality of protein-protein interaction. By a docking energy measure. The worse the outcome, the lower the score: 16.3, RMSD Root mean square deviation on scores about 16 marks approximately average distance among atoms weighted upon one another protein superposition. It shows how closely the docked conformation reflects the native conformation. RMSD: 2.2 Å. Z-Score: The Z-score judges the HADDOCK score against several random scores to determine whether the docking result is significant. Z-Score: 2.4 Binding Affinity to Control Antigens to validate the specificity and efficacy of the immunotoxins, we compared their binding affinities against control antigens, CD22 and CD34. (Figures 2 and 3 show the schematic binding interactions of the designed drugs to these antigens. CD22 Bindin. The researchers found that CD22 is a good target for therapy because it is taken up quickly into the cell with the investigational agents which resulted in increased uptake and cytotoxic activity. Moreover, there is a greater affinity as shown by improved docked scores for this ligand. It binds specifically to CD34+ T cells that are bright making it efficient choosing this molecule to carry toxins to those that bear them. When looking at different antigens it can be concluded that this one has more significant binding characteristics Variants that had intermediate affinity for EGFR had a wider

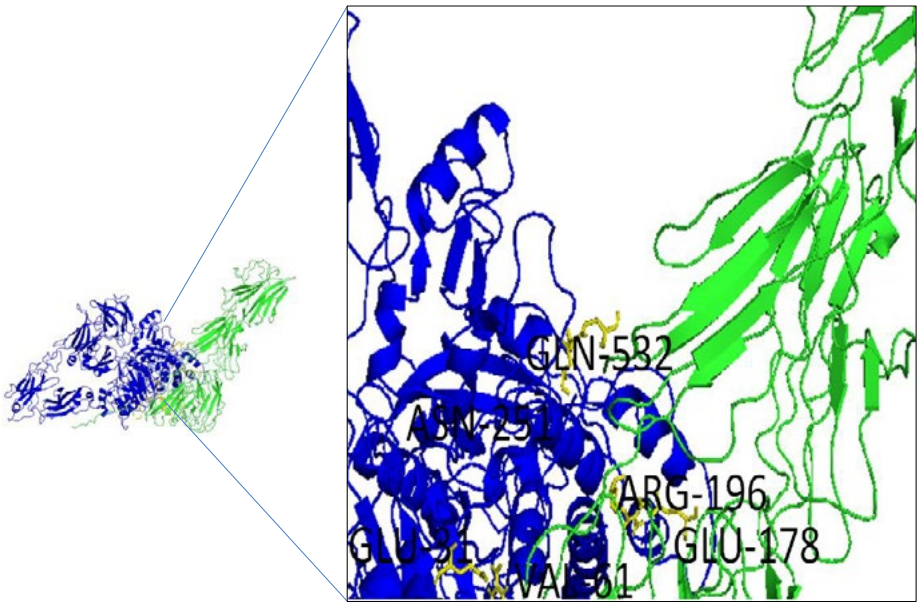

Fig 2. Schematic figure of binding of the designed drug to CD22 antigen

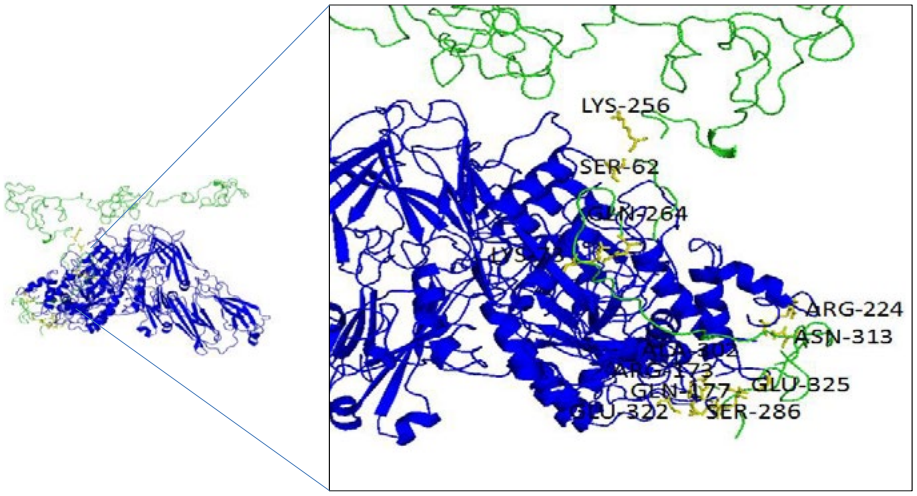

Fig 3. Schematic figure of binding of the designed drug to CD34 antigen

therapeutic window and could sustain higher maximum tolerated doses. The binding affinity: Optimal binding affinities are related to increased strength as well as higher cure rates. Trimeric Immunotoxin Targeting CEA: Its antitumor effects were significantly enhanced in human colorectal cancer xenografts compared to monomeric forms; thus, it underscores the significance of docking in immunotoxin optimization. Highly active trimeric forms have higher antitumor propensity; as detected by increased attachment affinity and cell killing. Several ways to use the computer for attaching antigen and protein toxins had been tried out to see if they might be effectively used together. Information Driven Docking (HADDOCK): Outperforms other programs because it uses CDRs and binding epitopes databases and other software in terms of performance and quality of the model. Complementarity between components of the interface; they can compute feasible starting attitudes (within 4.8 Å) displaying precise docking predictions thanks to their performance similar to that of known algorithms such as GSLIDE (5). The importance of optimizing affinity constants for a better therapeutic index in the efficacy and safety of immunotoxins became more apparent with these recent findings. This interpretation further highlights the significance of optimizing binding constants to improve the therapeutic, index, and margin of safety as well as decreasing immunogenicity levels. Based on the findings, immunotoxins hold great promise for the treatment of many types of cancer, including breast tumors, if they have optimal binding interactions. For the development of the targeted cancer therapy industry, such technologies must continue to evolve. By adjusting EGFR's bond ability, the therapeutic

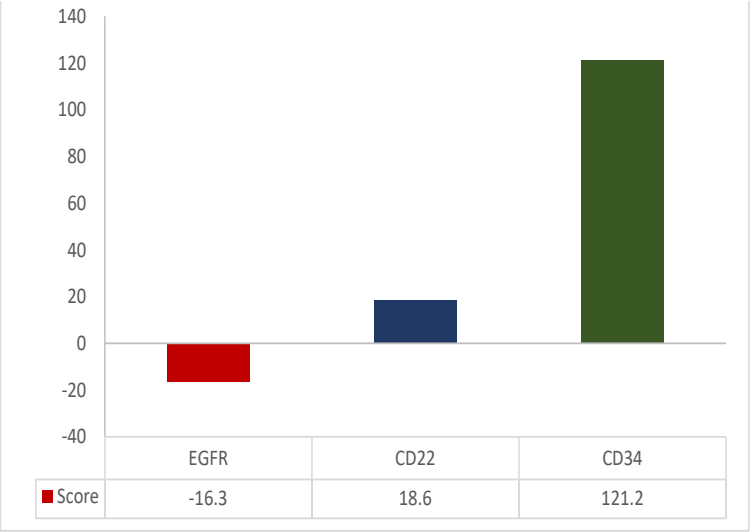

Fig4. Comparison of binding affinity of antigens with immunotoxin

interval of PE24 immunotoxin aimed toward EGFR-boosted cancers is extended. This research was about improving the therapeutic window by changing the bonding capability of anti-EGFR immunotoxins. The researchers produced numerous mutants that had varying reproducibility for EGFR and realized that some of these had better ranges of medication to toxicity than others in tumor models Intermediate affinity for example was associated with wider therapeutic windows and higher maximum doses than high-affinity tumors Therefore; it is known from the results that effectiveness of immunotoxins depends on an optimal binding strength this antigen. Immunoglobulin toxins' response rates will not be determined by anything but how they bond with respective antigens which are their targets. Immunotoxin effectiveness hinges mainly on the way it binds with an associated antigen, and it is in this context that a collection of analyses has been done. The more firmly immunotoxins are bound to cells' antigens, the greater their ability to kill these cells; nevertheless, sometimes exceptions do occur as a result of changes in bond strength between the particular toxin domains. Modifying affinity for binding can help to bring out these improvements thus enhancing immunotoxin efficacy which might reduce its side effects during its use in hospitals.

DISCUSSION & CONCLUSION

Cancer, which is a group of diseases characterized by abnormal cell proliferation, is second only to cardiovascular diseases as the leading cause of death among developed countries and third in developing ones. Over 25 million people worldwide are currently battling the condition, which adds up to more than 11 million fresh cases each year. Such dreadful statistics illustrate how dreadful this illness is: it can be described as an unnatural growth of body cells that tends to spread across different organs or tissues within an organism. Unlike harmless cancers, malignant tumors spread to other parts of the body. Humans have more than a hundred types of cancer which are associated with them. Cancerous cells grow within normal cells as either malignant or benign tumors. These growths are non-cancerous but typically benign and can be slow-growing like normal cells, however, their rapid division may lead to problems like swelling (which causes discomfort) or constricting pressure on adjacent organs as they grow too big for the space. Fast-growing cancerous growths, on the other hand, tend to spread to other parts of the body either through blood or lymphatic vessels where they form secondary tumors called metastases. Cancers are categorized according to the specific body tissues they affect; they are usually carcinoma of epithelial cells which account for a large proportion among elderly people about all types of cancers and we can also mention such ones as cancers of the lungs, prostate gland, or mammary gland. A malignant tumor is characterized by rapid multiplication without regulation and moving away from the place where it started. Human-like classifications include lymphatics, sarcomas are connected tissues or originate from mesenchyme outside of BM like nerves and muscle, and are the most widespread forms of cancer in children. The germ cells give rise to cancers such as those of testes and ovaries while embryonic cells account for pluripotent tumors found in different parts of the body. Blastomas arise from immature precursor cells (15-20), or embryonic tissue but without detailed sampling. Latin or Greek root combined with the suffixes carcinoma, sarcoma, or blastoma is typically used to label most cancers. For instance, though liver cancer could more accurately be referred to as hepatoblastoma, it is generally called hepatocellular carcinoma because it occurs in the liver;

similarly, while there are other names for adipose tissue tumors (cosmetic liposarcoma), people commonly say lipoma when speaking about fat cell tumors. Approximately 40,000 individuals perish yearly due to breast cancer which rates as one of the most frequently diagnosed malignancies in American women folk. Age-related death tolls differ depending on where individuals live – for instance, those who come from the North-Eastern part of the United States are more prone to dying from breast cancer than their Southern counterparts. It is estimated by analysts that before 2030 more than two million new cases of breast cancer will emerge every year mainly in underdeveloped areas, Immunohistochemistry and molecular approaches are employed in diagnosing breast cancer while the therapy involves traditional modes of treatment among others. Radiation, surgery, and chemotherapy are involved in traditional treatments, whereas hormone therapy, bacteriotherapy, and targeted treatments like cytotoxic, gene therapy, and immunotoxins are included in modern treatments. Nonetheless, traditional and new treatments still come with side effects such as nausea, immune suppression, and tissue damage. Diagnosing and treating cancer remains a significant challenge so The creation of new diagnostic and targeted therapeutic techniques is needed from bacteriotherapy, particularly with concern to the development of immunotoxins based on bacterial products gained prominence. Immunotoxins are hybrid proteins that contain a binding region, typically an antibody or any of its derivatives. The proteins also have a potent toxic part, which is usually an enzyme from plants or vegetables that binds specifically to an antigen on the surface of a cancer cell, killing it. Designing efficient immunotoxins has benefited significantly from intensive studies on bacterial poisons. We want to find out about new things that come from poison and harm cells but can be used as medicine to kill cancer cells. For this purpose, we are studying bacterial toxins made by dangerous germs and investigating how they are put together genetically and chemically for possible use as part of more suitable types of DNA that would destroy breast cancer cells when activated inside these dangerous organisms.

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Suggestions for further research

Optimizing Immunotoxin Design
Reducing Immunogenicity
Enhancing Internalization and Cytotoxicity
Exploring Novel Toxins
Clinical Trials and Translational Research
Tumor Microenvironment and Immune Modulation
Advanced Docking and Computational Methods
Patient-Specific Therapies.

Consent for publication

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Conflict of interest

No conflict of interest.

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