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Review Ariticle

Revolutionary Study in Antibody and Bacterial Mediated Immunotoxin for Breast Cancer Treatment

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Abstract: Many of this treatments for Breast cancer (BC-carcinoma), including surgery, radiation, and chemotherapy, haven't been effective in diminishing mortality rates, but bacterial mediated Antibody-immunotoxins has become one in all the best approaches to cancer treatment. Under these exceeding background, the present review, based on increasing mini report on bacterial mediated immunotoxins for breast cancer treatment. Various methodology suggest that, antibody therapies has been an central component in the association of malignant disease. Antibodies be capable of block the tumour growth factors or their specificity receptors and activate the immunological attack on the tumour microenvironment especially in breast cancer cells leads to tumor regression, and are used to deliver payloads such as radioisotopes, cytotoxic drugs or toxins. Immunotoxins are a new class of antitumour agents consisting of tumour selective ligands (generally mono clonal antibodies) linked to highly toxic protein molecules such as non-pathogenic bacterial species or programmed bacteria and take the advantage of the exquisite specificity of antibodies to selectively target drug delivery and the potency of toxins to kill the target cells. Currently, the attenuated bacteria as antitumor agents and vectors for gene directed enzyme prodrug or antibody conjugate therapy or immunotoxin have emerged as potential strategies.

Keywords: Bacterial Mediated Immunotoxin, Breast Cancer, HER-2, Trastuzumab and Chimeric Toxins

1. Introduction

Breast cancer (BC) is one in all the foremost prevalent sorts of cancer among women worldwide, which is acknowledged because the majority of distinguished reason for cancer mortality [1]. Whole genome expression studies using microarrays have led to classification of BC into five deferent subtypes of breast carcinomas based solely on clustered gene expression; luminal A (ER+ and/or PR+, HER2, low Ki-67), luminal B (ER+ and/or PR+, HER2/+, high Ki-67), HER2-overexpressing (ER+, PR+ and HER2+), basal-like (express markers of basal/myoepithelial cells), and normal breast-like (enriched in markers of adipose cells/normal mammary cells) [2].

The role of the Sonic hedgehog protein (Hh) pathway in BC tumorigenesis and progression, its prognostic role, and its value as a therapeutic target vary in line with the molecular and histological subtype of BC. Particularly, Hh signaling appears to be a vital mechanism in carcinoma with ER and PR-positive tumors and also Triple Nagative carcinoma TNBC [3-7]. An additional research explored that estrogen increases SHH and GLI1 expression resulting in activation of Hh signaling (determined by GLI1 nuclear translocation) and promotes invasiveness within the ER-positive T47D (HER2-) and BT-474 (HER2+) cells [8]. These results suggest a crosstalk of ER, HER-2 and also the Hh signaling pathways to extend invasiveness of ER-positive BC cells.

Many of this treatments for BC-carcinoma, including surgery, radiation, and chemotherapy, haven't been effective in diminishing mortality rates, but immunotherapy has become one in all the best approaches to cancer treatment. In an exceedingly model of human, HER- 2/neu (C) carcinoma (neu-transgenic mice), topical treatment with a TLR7 agonist, imiquimod, showed significant regression of spontaneous breast cancers.

Various reports suggest that, antibody therapies have been

acentral component in the association of malignant disease. Antibodies be capable of block the tumour growth factors or their specificity receptors and activate the immunological attack on the tumour microenvironment especially in breast cancer cells leads to tumor regression, and are used to deliver payloads such as radioisotopes, cytotoxic drugs or toxins. Immunotoxins are a new class of antitumour agents consisting of tumour selective ligands (generally mono clonal antibodies) linked to highly toxic protein molecules such as non-pathogenic bacterial species or programmed bacteria and take the advantage of the exquisite specificity of antibodies to selectively target drug delivery and the potency of toxins to kill the target cells. Currently, the attenuated bacteria as antitumor agents and vectors for gene directed enzyme prodrug or antibody conjugate therapy or immunotoxin have emerged as potential strategies. Under these exceeding background, the present review, based on increasing mini report on bacterial mediated immunotoxins for breast cancer treatment.

2. Monoclonal Antibodies

Monoclonal antibodies are man-made versions of immune system proteins (antibodies) that are designed to attach to a specific target. In this case, they attach to the HER2 protein on cancer cells, which can help stop the cells from growing.

Trastuzumab (Herceptin) is one of the humanized monoclonal anti-HER2 antibodies used for targeting HER2-positive breast cancer [9] Shown Figure 1. It shows better improvement rates during therapy than other treatments do, patients have demonstrated resistance to trastuzumab, even when combined with other chemotherapy drugs [10]. Based on the literatures, it is suggested that treatment with anti-HER2 antibody-targeted toxin is possibly more effective than the anti-proliferative antibody for patients [11]. Although, it has a small antibody fragment composed of heavy (VH) and light (VL) chains, which has to connected to one another through short flexible peptide linkers (about 10 - 25 amino acids). This has been used as immunotoxins for its low molecular weight (~ 1 kDa), high elasticity in chemical conjugation, low antigenicity, easy production, great tissue/cell penetration, and high biocompatibility, binding, and specificity. Trastuzumab (Herceptin) has been extended to transfer toxins, specifically to cancer cells.

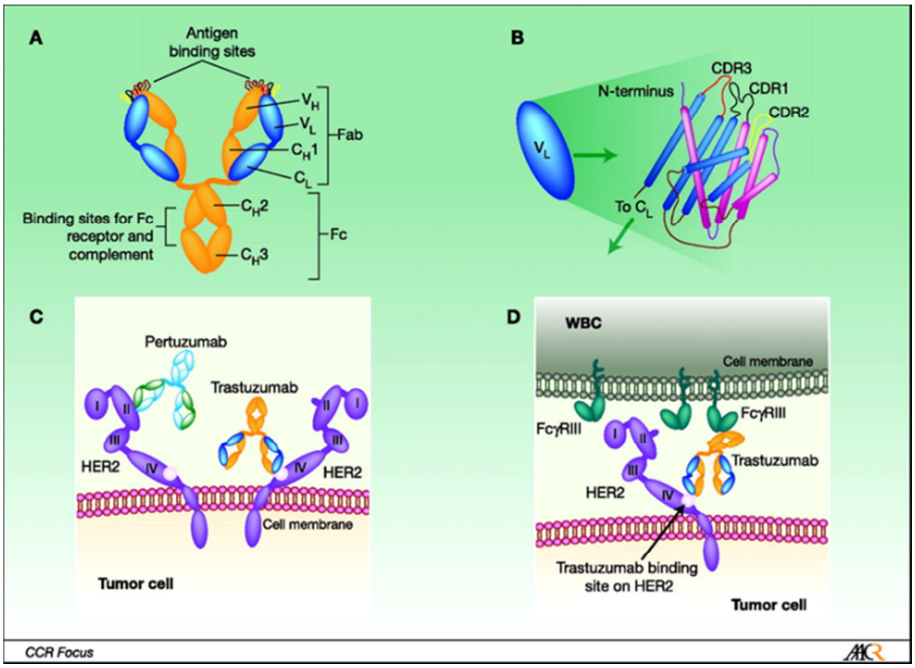

Figure 1. *Resistance to Trastuzumab in Breast Cancer.*

3. Bacteria in Cancer Therapy

The attenuated, genetically modified, non-pathogenic bacterial species and bacterial spores are also capable of inhibiting the growth of cancer. The most potential and promising strategy is bacteria based gene-directed antibody-bacterial toxin therapy. The German physicians W. busch& F. fehleisen, and American physician William coley, an Observed at 'coley's toxins'-Two heat killed bacterial species from *Salmonella pyogenes*and *serratiamarcescens*has been killed the cancer cells. Obligate anaerobes (e.g Clostridium and bifidobacterium) - target the anoxic area of tumor, and inhibit the growth of tumor. Facultative anaerobes (e.g. Salmonella and Escherichia) identify and penetrate tumors by detecting and chemotaxising towards small molecule gradients of serine, aspartate and ribose [12-15].

Clostridium novyi–NT (attenuated clostridium strain) produces toxicity in tumor even after deletion of genes coding for a lethal toxin. COBALT- Clostridium novyi-(combination bacteriolytic therapy)- NT spores were administered in combination with conventional chemotherapeutic agents like dolastatin-10, mitomycinC,vinorelbine and docetaxel. Bacillus Calmette Guerin (BCG), the most successful

bacterial agent so far is used specifically for the treatment of superficial bladder cancer. The Salmonella strain VNP20009 after deletion of two genes msb& purl results in its complete attenuation. The VNP20009 has been successfully investigated in phase 1 clinical trials in cancer patients [16, 17].

For delivering anticancer agents, cytotoxic peptides, therapeutics protein or produrg converting enzymes e.g. Salmonella, have been shown that when transfer of anti-angiogenic genes (thrombospondin and endostain) from salmonella, prevent the formations of new blood vessel and stopped the nutrient supply in tumor cell.

Cytosine deaminase (CD) have been successfully expressed in C. sporogenes and C. acetobutylicum and Salmonella vector has also been combined with CD and nitroreductase, and success has been observed *in vivo* and it is currently undergoing in phase 1 clinical trials [18].

Cyclomodulinsas a bacterial toxins that subvert the host eukaryotic cell cycle. E.g. 1. CNF (from *E.coli*)-cytotoxic necrosis factors, a cellcycle stimulator that triggers the G1 - S transition and induces DNA replication. E.g-2. CDTs (cytolethal distending toxins) found in several Gram- negative bacteria, for the cell-cycle inhibitor and E.g-3. Cif from enteropathogenic and enteroheamorrhagic*E.coli,* block mitosis and inhibit clonal expansion of lymphocytes.

4. Bacterial Toxins Binding to Surface Antigens

The Pseudomonas exotoxin A inhibit protein synthesis by catalytically ribosylate EF-2 and Clostridium perfringensenterotoxic (CPE) investigated for colon, breast, and gastric cancers due to its cytotoxic property. The Botulinum neurotoxin (BoNT) briefly opens tumors vessels, allowing more effective destruction of cancer by radiotherapy and chemotherapy. Some other toxins undergoing on research they are Alfa toxin from *Stapylococcousaureus*, AC-toxins from Bordetella pertussis, shiga like toxins - cholera toxin and various targeted immune-toxins are present in Figure 2.

Immunotoxin	Target	Targeting moiety	Toxic moiety	Tumor type	Reference
Erb38	erbB2/HER2	Anti-Her2/neu dsFv	PE38	Breast carcinoma	Choudhary et al.[82]
scFv(FRP5)-ETA	erbB2/HER2	Anti-Her2/neu scFv(FRP5)	ETA	Melanoma, breast, and colon	Choudhary et al.[82]
SS1P (SS1(dsFv)-PE38)	Mesothelin	Antimesothelin dsFv	PE38	Mesothelioma, ovarian, and pancreatic cancers	Hassan et al.[84]
LMB-1	Lewis Y	Anti-Lewis Y MAb	PE38	Adenocarcinoma	Pastan et al.[45]
LMB-7	Lewis Y	Anti-Lewis Y scFv(B3)	PE38	Adenocarcinoma	Pastan et al.[45]
LMB-9	Lewis Y	Anti-Lewis Y dsFv(B3)	PE38	Adenocarcinoma	Pastan et al.[13]
SGN-10	Lewis Y	Anti-Lewis Y dsFv(BR96)	PE40	Adenocarcinoma	Posey et al.[43]
OvB3-PE	Ovarian cancer	Murine MAb	PE	Ovarian carcinoma	Pai et al.[85]
TP40	EGFR	TGF-α	Modified PE40	Bladder cancer	Messing and Reznikoff[86]

EGFR: epidermal growth factor receptor; TGF: transforming growth factor.

Figure 2. *Immunotoxin and targeted cancer.*

5. Bacterial Toxin Conjugated to Ligand

The binding of toxins to cell-binding proteins. e.g Monoclonol antibodies or growth factors, cause regression in growth of cancer by targetting them into the specific sites on cancers. For example transferrin-Diphtheria toxin (DT), DT for epidermal growth factor (EGF) in brain tumor and metastatic carcinomas and IL4-PE against human gliboblastoma tumor and Genetically modified and recombinant toxins e.g chimeric toxins to be bio-conjugated to certain monoclonal antibodies have been shown potential tumor regression in breast cancer cell lines. The problems with bacterial therapy are coordinated with Toxicity, Infections, incomplete tumor lysis and DNA mutation.

6. The Flagellar Antigens-Flic from E.Coli as Bio-conjugate and Adjuvants

Another report showed that, flagellin has beenisolated from E.coli and characterized by the phase variation phenomenon, in which the controlled expression of several flagellin-encoding genes isolated from e.coli, salmonella typhi, psudomonos results in the expression of alternate flagellar antigens, originallycall as phase 1 (H1) and phase 2 (H2) flagellins, but currently referred to as FliC and FljB flagellins, severally [12]. FliC, its a structural fractional monetary unit of flagellar filaments, contribute to each virulence pathogenicity, and conjointly inflammatory responses activation in class hosts [13]. The necessary perform of flagellin (FliC) in microorganism motility, presents it as associate model candidate for innate immune recognition through act as adjuvants square measure agents that facilitate to extend, and suitably orient immune responses [19, 20].

Toll-like receptor 5 (TLR5) is a pattern recognition receptor, and FliC is its pathogen associated molecular pattern (PAMP), which stimulates host defense in a variety of organisms, including plants, insects, and mammals. This adjuvant property of FliC has been exploited by fusing polypeptides to FliC to render them antigenic. FliC is a unique PAMP because it harbors an antigenic hypervariable region, and a conserved domain which is involved in TLR5-dependent tumor

regression and systemic, mucosal pro-inflammatory, and adjuvant activities [21]. The FliC from Escherichia coli are the known paradigms for studies on flagellum structure-function, immunity, and TLR-5 signalling.

7. Antibody Conjugated Bacterial Immunotoxin for Breast Cancer Treatment

Breast cancer is recognized as the most frequent malignancy in the middle of female adults of various races and ages, and it is the second cause of cancer mortality (21). In particular, Sonic hedgehog protein (Hh) signaling appears to be a crucial mechanism in breast cancer with ER and PR-positive tumors and additionally Triple Nagative Breast Cancer TNBC [21-24]. An additional research confirmed that estrogen increases Sonic hedgehog protein (Hh) and GLI1 expression was most important to activation of Hh signaling (determined by GLI1 nuclear translocation) and promotes invasiveness in the ER-positive T47D (HER2-) and BT-474 (HER2+) cells [24]. These consequences suggest a crosstalk of ER, HER-2 and the Sonic hedgehog protein (Hh) signaling pathways to increase invasiveness of ER-positive BC cells (24).

According to abundant research and observations, it has been stated that conventional therapies for breast most cancer such as surgery, radiation, and chemotherapy have not decreased mortality rates [24]. Among subtypes of breast cancer, it is revealed that HER2-positive patients shows worse prognosis and shorter survival. Therefore, HER2 and Sonic hedgehog protein (Hh), an overexpressed surface receptor on breast cancer cells. Since it was selected for targeted therapy, and over the years, the survival of HER2-positive patients has increased (24). The first humanized monoclonal anti-HER2 antibody is trastuzumab (Herceptin). According to inquiries, it is declared that only 20% of patients with the overexpression of HER2 respond to trastuzumab [23, 24].

Nowadays, immunotoxin or antibody-antigen complex therapy has attracted great consideration for its specificity and effectiveness on cancer cells. Immunotoxins obtain their toxicity from a protein toxin and their specificity from an antibody [24].

Zhang et al. 2017; constructed two bacterialimmunotoxins based on trastuzumabscFv and a cytotoxic drug DM1 called T-SA1-HAS-DM1 and TSA2-HAS-DM1. T-SA1-HAS-DM1 showed potential anti-tumor activity and was distributed in xenograft models Shown Figure 3. The T-SA1-HSA. T-SA1-HAS consisted of VL and VH of trastuzumab, which was bound together by the GGGGSGGGGSGGGGS linker. Furthermore, the linker between scFv and HSA was GGSGG (23). Recently, Goleij et al. designed an immunotoxin that contained a scFv, whose VL and VH were bound together by the GGGGSGGGGSGGGGS linker. Their results showed that this scFv was stable and could bind to HER2 effectively [24].

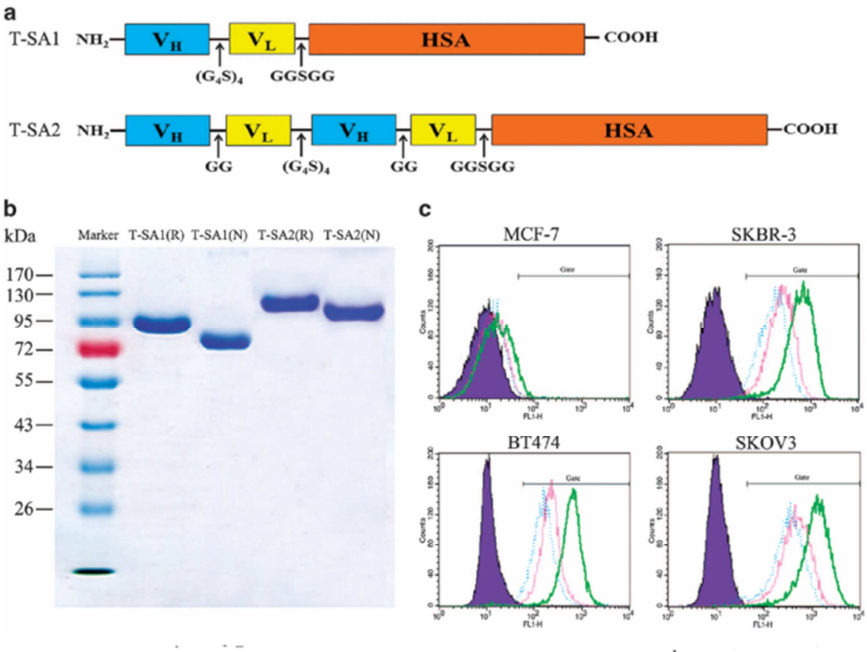

Figure 3. *Zhang et al. (2017) reported,ScFv + Bacterial Immunotoxin, a) Indicated the T-SAI and T-SA2 bacterial immunotoxin, b) Indicated the DNA quatification of the immune toxins and c) Indicated the resistance in various breast cancer cell line.*

The efficiency of an bacterial immunotoxin is dependent on successful endocytosis, which is based on scFv and receptor interaction and internalization of the receptor-bound immunotoxin [24, 25]. The immunotoxin-HER2 complex is internalized via receptor-mediated endocytosis. After internalization, the complex can be sorted in early endosomes or in multivesicular bodies. Finally, HER2 is recycled to the membrane and the immunotoxin can go to Golgi [24, 25]. The cleavage of cytolethal distending toxin (CDT) is initiated by the furin protease of the Golgi system;

then, it retrogrades to the endoplasmic reticulum (ER) and, ultimately, it is transported into the nucleus and leads to DNA damage [25]. In accordance with this pathway and based on the fact that furin is enriched in the Golgi complex and acts as a protease for protein cleavage. Weldon et al. designed immunotoxins based on Pseudomonas Exotoxin A that contained furin cleavage sites. Their study results showed the furin cleavage site is necessary for toxin activation in the Golgi apparatus and after scFv omission, the toxin can release to the cytoplasm.

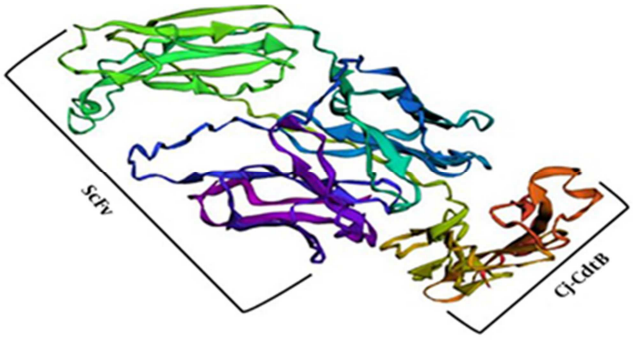

Figure 4. *Shown the AsmaVafadar, et al, (2020) reported, 3D-construct of HER-2 antibody of ScFv + bacterial conjucate-Cj-CdtB.*

Figure 5. *Shown the AsmaVafadar, et al, (2020) reported, 3D-Sequence construct of HER-2 antibody of ScFv + bacterial conjucate-Cj-CdtB.*

AsmaVafadar, et al 2020 reported, breast cancer is the most prevalent type of cancer among the female population, and about 15% to 20% ofpatients with breast cancer is human epidermal growth factor receptor 2 (HER2)-positive. The current cancer treatment methods such as surgery, radiation, and chemotherapy are not sufficiently effective in decreasing mortality rates; however, bacterial mediated immunotherapy is a novel approach in the treatment of cancer that is more efficient and less harmful to the body. Anti-cancer immunotoxins are chimeric molecules containing two parts, namely the immuno part, which is an antibody or a binding segment of antibody, and toxin part, which is a killer toxin molecule. In his study, sought to design a novel immunotoxin, including the anti-HER2 receptor, trastuzumab, derived from a single-chain variable fragment (scFv) with a connection to the functional part of bacterial *Campylobacter jejuni*cytolethal distending toxin (Cj-CdtB) Shown figures 4-5. The chimeric protein's physicochemical properties, solubility, and secondary structure were analyzed, using ProtParam, PROSO II, and GORV, respectively. A three-dimensional (3D) model was built, using I-TASSER and refined, usingGalaxyRefine. The model's structure was evaluated before and after refinement, using PROCHECK and RAMPAGE. The AlgPred server was employed to predict immunotoxinallergenicity, and mRNA stability was evaluated by RNAfold. Finally, the immunotoxin and HER2 were docked, using ZDOCK. Analysis showed that the chimeric protein could be a stable and soluble protein and the secondary structure of its parts would not change and the protein had a robust 3D structure that might have a stable mRNA structure and could bind to HER2 receptor: The designed bacterial mediated immunotoxin was a stable and soluble protein with the ability to bind to HER2 receptors, making it an appropriate immunotoxin candidate for breast cancer treatment.

8. Conclusion

Antibody therapies has been an central component in the association of malignant disease. Anti bodies be capable of block the tumor growth factors or their specificity receptors and activate the immunological attack on the tumor microenvironment especially in breast cancer cells leads to tumor regression, and are used to deliver payloads such as radioisotopes, cytotoxic drugs or toxins. Immunotoxins are a new class of antitumour agents consisting of tumour selective ligands (generally monoclonal anti bodies) linked to highly toxic protein molecules such as non-pathogenic bacterial species or programmed bacteriaand take the advantage of the exquisite specificity of antibodies to selectively target drug delivery and the potency of toxins to kill the target cells. Immunotoxins are tailored to get rid of their traditional tissue-binding domains by genetic engineering. The analysis of this aminoacid sequence of the region specific for immune-genecity and therefore the signal transduction mechanisms concerned within the interaction of immunotoxins with neoplasm cells can provide the clue for the event of best immunotoxins. Currently, the attenuated bacteria for antitumor agents and vectors for gene directed enzyme prodrug or antibody conjugate therapy or immunotoxin have emerged as potential strategies. The VNP20009 and TAPET-CD have been investigated successfully in phase 1 clinical trial. The Chemericimmunotoxins are also being investigated as future toxin-based anticancer therapies especially for breast cancer treatment.

References

[1] AsmaVafadar, et al.,InSilico Design and Evaluation of scFv-CdtB as a Novel Immunotoxin for Breast Cancer Treatment, (2020) Int J Cancer Manag., 13 (1): e96094, doi: 10.5812/ijcm.96094.

[2] Sørlie, T.; Perou, C. M.; Tibshirani, R.; Aas, T.; Geisler, S.; Johnsen, H.; Hastie, T.; Eisen, M. B.; van de Rijn, M.; Je_rey, S. S.; et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. USA (2001), 98, 10869–10874.

[3] Habib, J. G.; O'Shaughnessy, J. A. The hedgehog pathway in triple-negative breast cancer. Cancer Med. (2016), 5, 2989-3006.

[4] Colavito, S. A.; Zou, M. R.; Yan, Q.; Nguyen, D. X.; Stern, D. F. Significance of glioma-associated.

[5] Oncogene homolog 1 (GLI1) expression in claudin-low breast cancer and crosstalk with the nuclear factor kappa-light-chain-enhancer of activated B cells (NF_B) pathway. Breast Cancer Res. 2014, 16, 444.

[6] Goel, H. L.; Pursell, B.; Chang, C.; Shaw, L. M.; Mao, J.; Simin, K.; Kumar, P.; Vander Kooi, C. W.; Shultz, L. D.; Greiner, D. L.; et al. GLI1 regulates a novel neuropilin-2/_6_1 integrin based autocrine pathway that contributes to breast cancer initiation. EMBO Mol. Med. 2013, 5, 488–508.

[7] Bieche, I.; Lidereau, R. Genetic alterations in breast cancer. Genes Chromosom. Cancer 1995, 14, 227–251.

[8] Tao, Y.; Mao, J.; Zhang, Q.; Li, L. Overexpression of Hedgehog signaling molecules and its involvement in triple-negative breast cancer. Oncol. Lett. 2011, 2, 995–1001.

[9] Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. *J Control Release*. 2010; 146 (3): 264–75. doi: 10.1016/j.jconrel.2010.04.009.

[10] Liu Y, Xu J, Choi HH, Han C, Fang Y, Li Y, et al. Targeting 17q23 amplicon to overcome the resistance to anti-HER2 therapy in HER2+ breast cancer. *Nat Commun*. 2018; 9 (1): 4718. doi: 10.1038/s41467-018-07264-0.

[11] Pernas S, Tolaney SM. HER2-positive breast cancer: New therapeutic frontiers and overcoming resistance. TherAdv Med Oncol. 2019; 11: 1.7588359198335E+15. doi: 10.1177/1758835919833519.

[12] Cunningham A F, Khan M, Ball J, et al. Responses to the soluble flagellar protein FliC are Th2, while those to FliC on Salmonella are Th1. Eur J Immunol. 2004; 34: 2986-95.

[13] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006; 124: 783-801.

[14] Alaniz R C, Cummings L A, Bergman M A, et al. Salmonella typhimurium coordinately regulates FliC location and reduces dendritic cell activation and antigen presentation to CD4+ T cells. J Immunol. 2006; 177: 3983-93.

[15] Munoz N, Van Maele L, Marques J M, et al. Mucosal administration of flagellin protects mice from Streptococcus pneumoniae lung infection. Infect Immun. 2010; 78: 4226-33.

[16] Nempont C, Cayet D, Rumbo M, et al. Deletion of flagellin'shypervariable region abrogates antibody-mediated neutralization and systemic activation of TLR5-dependent immunity. J Immunol. 2008; 181: 2036-43.

[17] Souzaki, M.; Kubo, M.; Kai, M.; Kameda, C.; Tanaka, H.; Taguchi, T.; Tanaka, M.; Onishi, H.; Katano, M. Hedgehog signaling pathway mediates the progression of non-invasive breast cancer to invasive breast cancer. Cancer Sci. 2011, 102, 373–381.

[18] LanariC,Wargon V, Rojas P, Molinolo AA. Antiprogestins in breast cancer treatment: Are we ready? *EndocrRelat Cancer*. 2012; 19 (3): R35–50. doi: 10.1530/ERC-11-0378.

[19] Li SG, Li L. Targeted therapy in HER2-positive breast cancer. *Biomed Rep*. 2013; 1 (4): 499–505. doi: 10.3892/br.2013.95.

[20] Allahyari H, Heidari S, Ghamgosha M, Saffarian P, Amani J. Immunotoxin: A new tool for cancer therapy. *Tumour Biol*. 2017; 39 (2): 1.0104283176922E+15. doi: 10.1177/1010428317692226.

[21] Sokolova E, Guryev E, Yudintsev A, Vodeneev V, Deyev S, Balalaeva I. HER2-specific recombinant immunotoxin 4D5scFv-PE40 passes through retrograde trafficking route and forces cells to enter apoptosis. *Oncotarget*. 2017; 8 (13): 22048–58. doi: 10.18632/oncotarget.15833.

[22] Raja SM, Desale SS, Mohapatra B, Luan H, Soni K, Zhang J, et al. Marked enhancement of lysosomal targeting and efficacy of ErbB2-targeted drug delivery by HSP90 inhibition. *Oncotarget*. 2016; 7 (9): 10522–35. doi: 10.18632/oncotarget.7231.

[23] Guerra L, Teter K, Lilley BN, Stenerlow B, Holmes RK, Ploegh HL, et al. Cellular internalization of cytolethal distending toxin: A new end to a known pathway. *Cell Microbiol*. 2005; 7 (7): 921–34. doi: 10.1111/j.1462-5822.2005.00520.x.

[24] Takahashi S, Nakagawa T, Banno T, Watanabe T, Murakami K, Nakayama K. Localization of furin to the trans-Golgi network and recycling from the cell surface involves Ser and Tyr residues within the cytoplasmic domain. *J Biol Chem*. 1995; 270 (47): 28397–401. doi: 10.1074/jbc.270.47.28397.

[25] Weldon JE, Skarzynski M, Therres JA, Ostovitz JR, Zhou H, Kreitman RJ, et al. Designing the furin-cleavable linker in recombinant immunotoxins based on Pseudomonas exotoxin A. *Bioconjug Chem*. 2015; 26 (6): 1120–8. doi: 10.1021/acs.bioconjchem.5b00190.