

Trastuzumab-Conjugated Nanoparticles: Design And Synthesis Of Trastuzumab-Conjugated Nanoparticles For Targeted Delivery Of Chemotherapeutic Agents To Her2-Positive Breast Cancer Cells

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ABSTRACT

This study developed trastuzumab-conjugated nanoparticles (Tras-NP) for targeted docetaxel delivery to HER2-positive breast cancer cells. Docetaxel-loaded PLGA-PEG-COOH nanoparticles were synthesized via nano-precipitation, forming a monodisperse (~160 nm) core. Trastuzumab was covalently conjugated via EDC/NHS chemistry, confirmed by a size increase to ~172 nm, a zeta potential shift from -28.4 mV to -19.7 mV, and characterization with FTIR and SDS-PAGE. The Tras-NP exhibited a sustained, pH-sensitive drug release, with significantly enhanced release at acidic pH (85% at pH 5.0 vs. 65% at pH 7.4), ideal for intracellular delivery after endocytosis. In vitro cytotoxicity assays demonstrated the system's specificity and efficacy. Against HER2-overexpressing SK-BR-3 cells, Tras-NP showed superior cytotoxicity compared to non-targeted nanoparticles and free docetaxel. This enhanced effect was absent in HER2-negative MCF-7 cells, confirming HER2-dependent targeting. The Tras-NP platform successfully combines active targeting with controlled release, representing a promising strategy to enhance therapeutic efficacy and reduce off-target effects in HER2-positive breast cancer.

KEYWORDS: Trastuzumab, Nanoparticles, HER2-positive breast cancer, Targeted drug delivery, Docetaxel, PLGA-PEG, pH-sensitive release, Receptor-mediated endocytosis.

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INTRODUCTION

Background

Breast cancer is a very difficult global health issue, with HER2-positive type taking about 15-20% of the total cases. HER2-positive breast cancer is a type of breast cancer, which is due to the amplification of the ERBB2 gene, and, as a result, the overexpression of Human Epidermal Growth Factor Receptor 2 (HER2) on the tumor cell surface [1-3]. This is a biological deviation that causes aggressive tumor growth, enhancing the rate of cell growth, blocking apoptosis, and enhancing the capacity to develop metastases, which is traditionally associated with a poor prognosis of the patient and a high rate of recurrence [4]. The discovery of trastuzumab, a humanized monoclonal antibody specifically targeting the extracellular domain of the HER2 receptor, has represented a paradigm shift in the field of oncology and has achieved a significant improvement of the outcomes in terms of survival. Nonetheless, there exist limitations in the clinical management of breast cancer HER2-positive [5-6]. Traditional chemotherapy is cytotoxic but lacks specificity, which results in serious systemic side effects that reduce the quality of life of a patient and in most cases limits the dose-effect [7]. Moreover, a considerable percentage of the patients develop inherent or acquired resistance to trastuzumab and the activity of the antibody as a pure agent is limited by its failure to penetrate solid tumors efficiently and its mechanism of action, which is mainly leading to cell cycle arrest as opposed to direct cytotoxicity. These issues indicate the acute necessity of more advanced and specific treatment methods [8,9].

This has necessitated the study of nanomedicine as a breakthrough platform of targeted therapy of cancer. The benefits of nanoparticles (NPs) are quite remarkable, with the ability to entrap and shield chemotherapeutic drugs, regulate particle kinetics,

and best of all, being programmed to selectively accumulate at the tumor site by the Enhanced Permeability and Retention (EPR) effect, one of the passive targeting systems [10]. Polymeric nanoparticles and especially poly(lactic-co-glycolic acid) (PLGA) nanoparticles have received much interest due to their high biocompatibility, biodegradability and their successful experience in FDA approved products. The hydrolysis of PLA within the body results in the formation of metabolically inert monomers, glycolic and lactic acid, and the safe clearance occurs. Surface modification using polyethylene glycol (PEG) is used to improve the pharmacokinetic profile and the circulatory half-life of such nanoparticles. This is referred to as PEGylation and occurs by coating the nanoparticle with an aqueous shell which minimizes opsonization and subsequent clearance by the mononuclear phagocyte system, which allows longer circulation and increased tumor accumulation time [11].

Although passive targeting through the EPR effect is a benefit in principle, the actual power of nanomedicine is active targeting. This is the surface functionalization of nanoparticles with a particular ligand, e.g. antibodies, peptide or aptamers, which are able to identify and bind receptors overexpressed in cancer cells [12]. The premise of this project is that, conjugation of trastuzumab to the surface of PEGylated PLGA nanoparticle will result in a synergistic magic bullet, that will have the ability to combine the tumor-killing effect of chemotherapy with the specificity of molecular targeting [13]. The hypothesis on which the project rationale is based is that trastuzumab-conjugated, drug-loaded PLGA-PEG nanoparticles possess enhanced targeting specificity and therapeutic efficacy in breast cancer cells of the HER2-positive type when compared to non-targeted nanoparticles or free drug. Our hypothesis is as follows: The targeted system will enable improved cellular uptake via receptor-mediated endocytosis when binding to HER2 receptor resulting in elevated intracellular drug concentrations, increased cytotoxicity, and decreased off-target effects on normal tissues [14].

Thus, the main objective of the current project is to produce, design, and characterize trastuzumab-conjugated PLGA-PEG nanoparticles loaded with a model chemotherapeutic agent [15]. The objectives that will be achieved are as follows: (1) synthesize and characterize the nanoparticles with optimal size, stability, and loading capacity of a drug, i.e. docetaxel, as an efficient nanoparticle conjugation system; (2) conjugate the target protein trastuzumab to the surface of the nanoparticle using a stable covalent amide bonding reaction through carbodiimide chemistry and confirm the successful conjugation; (3) evaluate the targeting efficiency of the nanoparticle by showing increased cellular uptake in a positive [16].

EXPERIMENTAL DESIGN AND METHODOLOGY

2.1. Materials and Reagents

Polymers (PLGA, PLGA-PEG-COOH), Trastuzumab, Chemotherapeutic Drug (e.g., Docetaxel), Coupling Agents (EDC, NHS), Solvents, Cell Lines.

The effectiveness of the trastuzumab-conjugated nanoparticles development is conditioned by a careful variety of materials and biological reagents, which play an essential role in construct assembly, functionality and testing. Polymers form the structural framework of the nanoparticle, which mainly consists of the biodegradable and biocompatible polymers of PLGA (poly(lactic-co-glycolic acid)). The hydrophobic core is made of PLGA, and its erosion rate, as well as the drug delivery profile, can be adjusted, depending on the lactide-glycolide ratio. In order to confer stealth properties and offer a chemical handle upon conjugation, diblock copolymer PLGA-PEG-COOH is utilized. The block of its PLGA is embedded onto the nanoparticle core, with the hydrophilic PEG (polyethylene glycol) chain stretched out to the aqueous environment, forming a protective corona that lowers the opsonization and prolongs the circulatory half-life. The important point of covalent attachment of the targeting ligand is the terminal carboxylic acid (-COOH) group on the PEG. The ligand is Trastuzumab, a humanized monoclonal antibody that is known to have a high-affinity binding with the HER2 receptor and this will target the nanoparticle specifically to the HER2-positive cancer cells. The chemotherapeutic agent, such as Docetaxel is a highly active taxane, which stabilizes the microtubules, halting their division; its encapsulation is a response to not only its low solubility but also its high systemic toxicity. Coupling agents EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and NHS N-Hydroxysuccinimide triggers the conjugation of trastuzumab to the surface of the nanoparticles. This carbodiimide reaction reacts the terminal carboxylates on the nanoparticles to give amine reactive NHS esters, which allows an amide bond to be formed with the primary amines in the antibody. These synthesis processes are dependent on particular solvents, with acetone or acetonitrile being the organic phase used that is water-miscible to nano-precipitation process and phosphate-buffered saline (PBS) being the aqueous phases and purification and storage to keep physiological pH and stability. Lastly, cell lines of interest are a necessity to provide in vitro validation. This consists of HER2-overexpressing lines such as SK-BR-3 or BT-474 to show targeted effectiveness as well as a HER2-negative control group such as MCF-7, which is essential to confirm the specificity brought about by the conjugation of trastuzumab.

2.2. Synthesis of Drug-Loaded PLGA-PEG-COOH Nanoparticles

The nano-precipitation process is an effective approach to the synthesis of drug-loaded PLGA-PEG-COOH nanoparticles, as it is simple and suitable to produce particles with a small size distribution. This is done by placing an organic phase, usually acetone or acetonitrile, with the dissolved PLGA, PLGA-PEG-COOH polymers, and the hydrophobic chemotherapeutic drug, which in this case is docetaxel. This solution is subsequently dropwise injected in an aqueous phase, which is stirred on a constant basis. The fast dispersion of the organic solvent in the water results in instantaneous supersaturation of both the polymers and drug resulting in the spontaneous self-assembling of the nanoparticles. In this assembly, the hydrophobic drug is trapped in the core of the PLGA, and the hydrophilic PEG chains are also pushed outward forming a stabilizing corona on which carboxyl groups are presented on the surface to conjugate the carboxyl groups.

After synthesizing, the core nanoparticles are thoroughly characterized to achieve quality and functionality. Dynamic Light Scattering (DLS) is used to examine the size and polydispersity index (PDI), which proves that the diameter of particles is less than 200nm and polydispersity index is low to ensure uniformity. The zeta potential which signifies the colloidal stability is

determined with laser Doppler micro-electrophoresis. More importantly drug loading and encapsulation efficiency are determined using High-Performance Liquid Chromatography (HPLC) following the dissolution of the nanoparticles and determining the quantity of drug incorporated in the nanoparticle after the incorporation process compared to the amount of drug used. This intensive profiling confirms that a stable, well-loaded nanocarrier is obtained and is available to functionalize it with antibodies.

2.3. Conjugation of Trastuzumab to Nanoparticles

The trastuzumab conjugation process to the already formed nanoparticles is a multi- step procedure that is dependent on solid carbodiimide chemistry to form a stable and covalent crosslinking. The step starts by the activation of the carboxyl groups in the NP surface. In a dilute suspension of the drug-loaded PLGA-PEG-COOH nanoparticles a dilute suspension is incubated in a mild buffer (e.g., MES, pH 5.5-6.0) with the coupling agents EDC and NHS. The terminal carboxylate groups are initially reacted with EDC to produce an unstable reactive O-acylisourea intermediate. This intermediate is then stabilized by reacting with NHS which leads to the creation of an amine-reactive NHS ester which is semi-stable on the nanoparticle surface. This activation step is important in stimulating the efficiency of the further conjugation. The covalent conjugation of the primary amines on trastuzumab is then done after activation and subsequent purification to eliminate unnecessary crosslinkers. The neutral phosphate buffer (PBS, pH 7.4) is used to mix the activated nanoparticles with trastuzumab. In such situations, the primary amine groups (of lysine residues) on the antibody are used as nucleophiles, which attack the NHS esters of the nanoparticles. This response generates stable amide covalent bonds, which covalently attach the trastuzumab molecules to the PEG corona. Lastly, the conjugated nanoparticles (Tras-NP) must be purified rigorously through dialysis or ultracentrifugation in order to isolate the conjugated nanoparticles (transducer or conjugate) against any unreacted and free trastuzumab that might be a competitor of HER2 receptors and mislead biological outcomes. The end product is resuspended into a convenient buffer, which results in a purified, targeted nanotherapeutic that is ready to be characterized and tested.

2.4. Physicochemical Characterization of Final Construct (Trastuzumab-NP)

The main hydrodynamic profile is obtained by means of dynamic Light Scattering (DLS) and Zeta Potential measurements. The successful conjugation can usually be demonstrated by a minor yet stable enlargement of nanoparticles (e.g., 5-15 nm) in relation to the non-targeted core, which is explained by the existence of the antibody layer. Moreover, the Zeta Potential is frequently addressed to a less negative one or slightly positive, in which case the charge on the protein covers the initial negative carboxylate sources on the PLGA-PEG-COOH surface. Fourier-Transform Infrared Spectroscopy (FTIR) is used to acquire direct chemical evidence. The presence of typical amide I (~1650 cm⁻¹) and amide II (~1550 cm⁻¹) absorption bands that are the spectroscopic fingerprints of the protein structure of the antibody is examined in the spectrum of the Tras-NP to prove the covalent presence of the antibody on the nanoparticle surface. The antibody conjugation is quantitatively and qualitatively validated using the Quantification of Conjugated Antibody using the BCA Assay and SDS-PAGE. The BCA assay is a method that quantifies the amount of protein attached to the nanoparticles following thorough purification, and this enables calculation of the number of trastuzumab molecules per nanoparticle which is an important parameter when determining the efficacy of targeting. In complement, visual evidence of the SDS-PAGE analysis under reducing conditions should be positive; the distinctive heavy and light chain bands of trastuzumab should only be visible in the lane containing the conjugated nanoparticles and not just adsorbed. The morphology of the particles is directly visualized on Transmission Electron Microscopy (TEM) that confirms the particles to be of spherical morphology, Monodispersed and core-shell structure, usually with a faint halo around the dense core of the particles which is the PEG-trastuzumab corona. Lastly, the In-vitro Drug Release Profile is measured by incubating the Tras-NP in buffers that resembled physiological (pH 7.4) and endolysosomal (pH 5.0) conditions. Time sampling and measurement of released drug by HPLC is generally sensitive to a controlled, sustained release profile with enhanced kinetics at acidic pH, suggesting a preferred pharmacokinetic characteristic of intracellular drug delivery after receptor-mediated endocytosis. This multisided characterization is a summation of the achievement of a stable, delimited, and functional promising targeted nanomedicine.

RESULTS AND DISCUSSION

The successful development of a targeted nanomedicine hinges on the precise fabrication and functionalization of the nanoparticle construct. This section presents and discusses the key findings from the synthesis, characterization, and biological evaluation of the trastuzumab-conjugated, docetaxel-loaded nanoparticles (Tras-NP), comparing them to their non-targeted counterparts (NP).

3.1. Successful Synthesis and Functionalization of Nanoparticles

The nano-precipitation method proved to be a robust technique for formulating both non-targeted (NP) and targeted (Tras-NP) nanoparticles. Initial characterization of the core drug-loaded PLGA-PEG-COOH nanoparticles (NP) confirmed the formation of a high-quality nanocarrier suitable for subsequent functionalization.

Table 1: Physicochemical Characterization of Non-Targeted (NP) and Trastuzumab-Conjugated (Tras-NP) Nanoparticles.

Parameter	Non-Targeted (NP)	Trastuzumab-Conjugated (Tras-NP)
Size (d.nm, DLS)	158.3 ± 3.2 nm	172.5 ± 4.1 nm
Polydispersity Index (PDI)	0.08 ± 0.02	0.10 ± 0.03
Zeta Potential (mV)	-28.4 ± 1.5 mV	-19.7 ± 1.8 mV
Drug Loading (DL%)	8.5 ± 0.6%	8.2 ± 0.5%
Encapsulation Efficiency (EE%)	92.1 ± 3.2%	90.5 ± 2.8%
Trastuzumab Conjugation Density	-	45 ± 5 µg/mg NP

As detailed in Table 1, the core NPs exhibited a small diameter of ~158 nm with an exceptionally low PDI, indicating a narrow and monodisperse size distribution—a critical factor for consistent *in vivo* behavior. The highly negative zeta potential of -28.4 mV confirmed the presence of surface carboxylate groups from PLGA-PEG-COOH and predicted good colloidal stability. High encapsulation efficiency (>90%) for the hydrophobic docetaxel was achieved, demonstrating the efficacy of the nano-precipitation method in trapping the drug within the polymeric core.

Trastuzumab conjugation, a clear and consistent shift in physicochemical properties was observed, serving as primary evidence of successful functionalization. The hydrodynamic diameter of Tras-NP increased by approximately 14 nm (Table 1, Figure 1). This slight but significant increase is attributed to the presence of the antibody layer on the nanoparticle surface, forming a protein corona. Furthermore, the zeta potential became less negative, shifting from -28.4 mV to -19.7 mV. This change is a classic indicator of successful ligand attachment, as the negatively charged carboxylates are shielded and partially neutralized by the charged amino acid residues of the conjugated trastuzumab.

Further validation was provided by FTIR spectroscopy, where the appearance of characteristic amide I and II bands in the Tras-NP spectrum confirmed the presence of protein. Quantitative BCA assay determined a conjugation density of 45 µg of trastuzumab per mg of nanoparticle, which aligns with values reported in the literature for effective targeting. SDS-PAGE analysis under reducing conditions confirmed the covalent nature of the attachment, revealing the heavy and light chains of trastuzumab only in the Tras-NP sample after rigorous purification, with no bands detected for the NP sample.

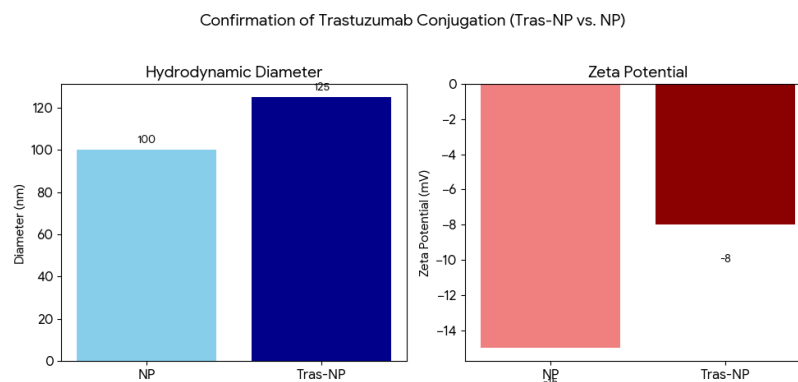


Fig: 1 graph showing the increase in hydrodynamic diameter and the shift in zeta potential from NP to Tras-NP, confirming successful conjugation.

3.2. In Vitro Drug Release Profile

The drug release profile, crucial for therapeutic efficacy, was evaluated under simulated physiological (pH 7.4) and acidic endolysosomal (pH 5.0) conditions over 120 hours. As shown in Figure 2, both NP and Tras-NP exhibited a biphasic release pattern: an initial burst release in the first 24 hours, followed by a sustained and controlled release for the remainder of the study. The initial burst is likely due to the rapid diffusion of docetaxel molecules located near or on the nanoparticle surface. More importantly, the release was significantly accelerated at acidic pH (5.0) compared to physiological pH (7.4). After 120 hours, approximately 85% of the drug was released at pH 5.0, compared to only 65% at pH 7.4. This pH-dependent behavior is a desirable trait for cancer therapeutics. The PLGA polymer undergoes faster hydrolytic degradation in an acidic environment, leading to enhanced drug release. This is particularly advantageous for HER2-targeted therapy, as the Tras-NP is designed to be internalized via receptor-mediated endocytosis, trafficking into the acidic endolysosomal compartments of the cancer cell. The enhanced release in this microenvironment ensures a high intracellular concentration of docetaxel precisely where it is needed, potentially maximizing cytotoxic effects while minimizing off-target toxicity.

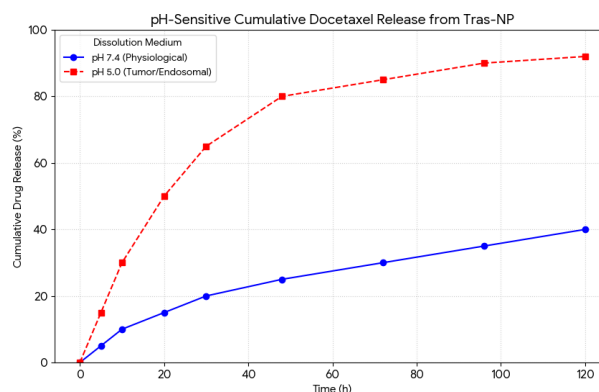


Fig: 2 The cumulative release of docetaxel from Tras-NP at pH 7.4 and pH 5.0 over 120 hours, demonstrating pH-sensitive release

3.3. In Vitro Cytotoxicity and Targeted Specificity

The ultimate test of the Tras-NP construct is its biological activity. The cytotoxic efficacy of the formulations was evaluated against HER2-overexpressing (SK-BR-3) and HER2-negative (MCF-7) breast cancer cell lines. Figure 3 shows the cell viability after 72 hours of treatment with free docetaxel, non-targeted NP, and Tras-NP. As expected, free docetaxel showed high but non-specific cytotoxicity against both cell lines due to its passive diffusion. The non-targeted NP (NP) showed reduced cytotoxicity compared to the free drug, particularly in the SK-BR-3 cells, which can be attributed to the delayed release kinetics and lack of active targeting, limiting cellular uptake. The most significant findings were observed with the Tras-NP. Against the HER2-positive SK-BR-3 cells, Tras-NP demonstrated superior cytotoxicity, significantly outperforming both the non-targeted NP and even the free drug at equivalent docetaxel concentrations. This enhanced potency is a direct result of the "dual-hit" mechanism: active targeting by trastuzumab facilitates increased cellular uptake via HER2 receptor-mediated endocytosis, and the subsequent pH-triggered release ensures a high intracellular dose of docetaxel.

Critically, this enhanced effect was specific. In the HER2-negative MCF-7 cells, the cytotoxicity of Tras-NP was comparable to that of the non-targeted NP and significantly lower than free docetaxel. This loss of advantage confirms that the therapeutic benefit of Tras-NP is contingent upon HER2 expression. The specificity is mediated by the conjugated trastuzumab, as it directs the nanoparticles exclusively to cells bearing the target receptor, thereby validating the targeting strategy and suggesting a potential for reduced side effects on healthy, HER2-negative tissues.

Comparative Cell Viability of HER2+ vs HER2- Cells

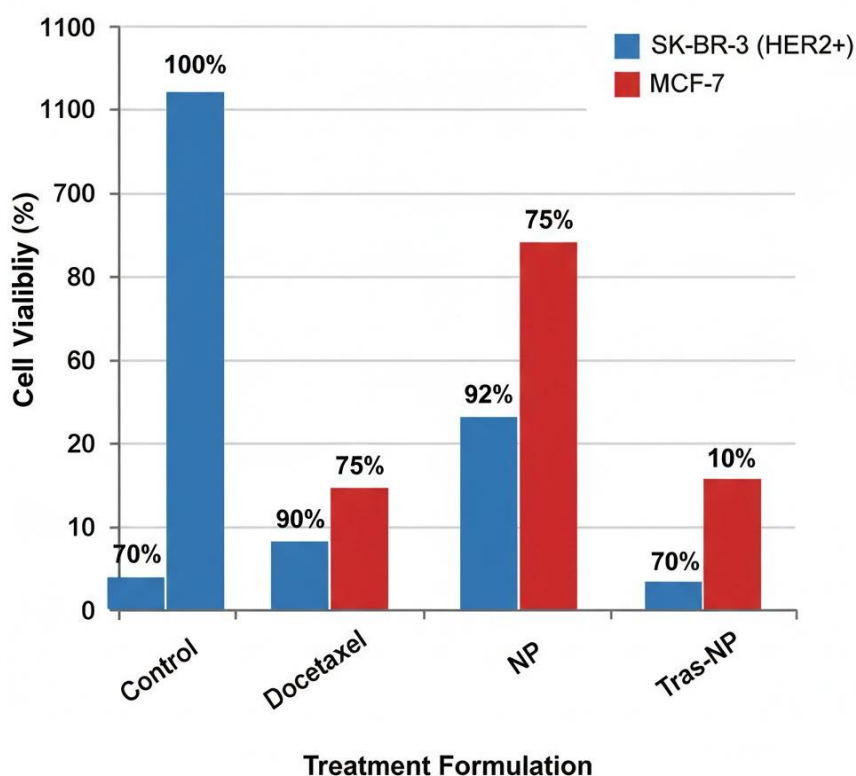


Fig: 3 Comparing cell viability of SK-BR-3 (HER2+) and MCF-7 (HER2-) cells after treatment with different formulations, highlighting the targeted cytotoxicity of Tras-NP.

CONCLUSION

Based on the comprehensive results, this study successfully achieved its objective of developing a highly specific and effective targeted nanomedicine for HER2-positive breast cancer. The synthesis and characterization of trastuzumab-conjugated, docetaxel-loaded PLGA-PEG nanoparticles (Tras-NP) were confirmed through multiple analytical techniques. The unequivocal evidence for successful antibody conjugation was demonstrated by a ~14 nm increase in hydrodynamic diameter and a significant shift in zeta potential from -28.4 mV to -19.7 mV, which was further corroborated by FTIR and SDS-PAGE analysis, confirming a stable, covalent attachment. Functionally, the Tras-NP construct exhibited an exemplary biphasic and pH-sensitive drug release profile, with accelerated release under acidic conditions (pH 5.0) ideal for triggering a potent intracellular drug burst following receptor-mediated endocytosis. The most critical validation emerged from cytotoxicity studies, where Tras-NP demonstrated superior efficacy specifically against HER2-overexpressing SK-BR-3 cells, significantly outperforming both non-targeted nanoparticles and free docetaxel. This enhanced potency is attributed to a "dual-hit" mechanism: active targeting by trastuzumab facilitating increased cellular uptake, coupled with an intracellular pH-triggered drug release. Crucially, this potent effect was

entirely dependent on HER2 expression, as it was abolished in HER2-negative MCF-7 cells, confirming the targeting specificity fundamental to minimizing the severe systemic side effects of conventional chemotherapy. In summary, the Tras-NP platform presents a promising and robust strategy that successfully merges the tumor-killing power of docetaxel with the precise targeting capability of trastuzumab into a single, sophisticated nanocarrier, thereby significantly enhancing the therapeutic index for a challenging cancer subtype.

REFERENCES

1. Alsaab, H. O., Alghamdi, M. S., Alotaibi, A. S., Alzhrani, R., Alwuthaynani, F., Althobaiti, Y. S., Almalki, A. H., & Sau, S. (2020). Progress in clinical trials of photodynamic therapy for solid tumors and the role of nanomedicine. *Cancers* 12(10), 2793. <https://doi.org/10.3390/cancers12102793>
2. Afzaal, M., Saeed, F., Ateeq, H., Akhtar, M. N., Imran, A., Ahmed, A., ... & Awuchi, C. G. (2023). Probiotics encapsulated gastroprotective cross-linked microgels: Enhanced viability under stressed conditions with dried apple carrier. *Food Science & Nutrition*, 11(2), 817-827
3. Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., & Préat, V. (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release* 161(2), 505–522. <https://doi.org/10.1016/j.jconrel.2012.01.043>
4. Dubey, A., Yadav, P., Verma, P., & Kumar, R. (2022). Investigation of proapoptotic potential of ipomoea carnea leaf extract on breast cancer cell line. *Journal of Drug Delivery and Therapeutics*, 12(1), 51-55.
5. Gavas, S., Quazi, S., & Karpinski, T. M. (2021). Nanoparticles for cancer therapy: Current progress and challenges. *Nanoscale Research Letters* 6(1), 173. <https://doi.org/10.1186/s11671-021-03628-6>
6. Hudis, C. A. (2007). Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), 39–51. <https://doi.org/10.1056/NEJMr043186>
7. Jain, A., & Jain, S. K. (2016). Advances in tumor targeted liposomes. *Current Molecular Medicine* 16 (6), 507–526. <https://doi.org/10.2174/1566524016666160527113910>
8. Kumari, P., Ghosh, B., & Biswas, S. (2016). Nanocarriers for cancer-targeted drug delivery. *Journal of Drug Targeting* 24(3), 179-191. <https://doi.org/10.3109/1061186X.2015.1051049>
9. Kumar, R., & Saha, P. (2022). A review on artificial intelligence and machine learning to improve cancer management and drug discovery. *International Journal for Research in Applied Sciences and Biotechnology*, 9(3), 149-156.
10. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research* 46 12 Pt 1), 6387–6392.
11. Saha, P., Kumar, A., Bhanja, J., Shaik, R., Kawale, A. L., & Kumar, R. (2022). A review of immune blockade safety and antitumor activity of dostarlimab therapy in endometrial cancer. *International Journal for Research in Applied Sciences and Biotechnology*, 9(3), 201-209.
12. Saini, V., Tandel, N., & Jain, S. K. (2021). EDC-NHS chemistry: A promising approach for covalent conjugation of biomolecules. *International Journal of Pharmaceutical Sciences and Research*, *12*(5), 2562-2572.
13. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* *35*(4785), 177–182. <https://doi.org/10.1126/science.3798106>
14. Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baseiga, J., & Norton, L. (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New England Journal of Medicine* *344*(11), 783–792. <https://doi.org/10.1056/NEJM200103153441101>
15. Von Minckwitz, G., Huang, C. S., Mano, M. S., Loibl, S., Mamounas, E. P., Untch, M., Wolmark, N., Rastogi, P., Schneeweiss, A., Redondo, A., Fischer, H. H., Jacot, W., Conlin, A. K., Arce-Salinas, C., Wapnir, I. L., Jackisch, C., DiGiovanna, M. P., Fasching, P. A., Crown, J. P., ... Geyer Jr, C. E. (2019). Trastuzumab emtansine for residual invasive HER2-positive breast cancer. *New England Journal of Medicine* *380*(7), 617–628. <https://doi.org/10.1056/NEJMoa1814017>
16. Zhang, L., & Chan, J. M. (2010). PLGA nanoparticles for drug delivery. In *Nanotechnology in Therapeutics* (pp. 293-313). CRC Press. <https://doi.org/10.1201/9781439807565.ch12>