

Preclinical Evaluation of Targeted Nanoparticle-Based Drug Delivery in Triple-Negative Breast Cancer

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Article Info	ABSTRACT
Article history: Received : 18.01.2025 Revised : 20.02.2025 Accepted : 22.03.2025 Keywords: Triple-Negative Breast Cancer (TNBC); Nanoparticle Drug Delivery; Targeted Therapy; Preclinical Study; Doxorubicin; Ligand Functionalization; Tumor Targeting; In Vivo Biodistribution; Chemotherapy; Drug Resistance.	Triple negative breast cancer (TNBC) is a special subtype of breast cancer that is currently not novelized, i.e. has no estrogen, progesterone and HER2 receptors, and therefore is difficult to be treated as well as has poor clinical outcomes. A preclinical evaluation with such a targeted nanoparticle based drug delivery system is presented in this study to enhance the therapeutic efficacy of TNBC with minimal systemic toxicity. Physicochemical properties of ligand functionalized nanoparticles with the encapsulation of doxorubicin were developed and evaluated for drug release kinetics and cellular uptake are presented. Targeted nanoparticles enhanced killing of TNBC cells versus free drug and non targeted controls in cytotoxicity assays of TNBC cell lines (MDA MB 231). These studies performed in TNBC xenograft mouse models show in vivo biodistribution and therapeutic efficacy with preferential tumor accumulation and tumor volume reduction. Off target organ toxicity was confirmed and reduced by histopathological analysis. The implications of these findings are the potential of targeted nanoparticle platform for enhancing the therapeutic index of chemotherapeutic agents in TNBC and their potential clinical translation.

INTRODUCTION

Triple negative breast cancer, TNBC, is approximately 10–20% of all breast cancers, it is categorized in cancer by its invisibility of 'Estrogen Receptor (Er)' 'Progesterone Receptor (Pr)' And 'Human Epidermal Growth Factor Receptor 2 (Her2)'. Since there are no known molecular targets for these receptors, hormonal and HER2 directed therapies are ineffective and chemotherapy is the only systemic treatment of choice. Nevertheless, the poor prognosis among TNBC patients as well as a high recurrence and metastasis rate necessitate more effective and targeted therapeutic strategies against this disease. Therefore, nanoparticle based drug delivery systems have become promising alternatives of conventional chemotherapy by overcoming of selective drug delivery, better pharmacokinetics and diminished systemic toxicity. Targeting ligands functionalized on nanoparticles, e.g., peptides,

antibodies, aptamers, favor accumulation of the nanoparticles in tumor tissues to maximize their therapeutic efficiency and minimize the off target effects. Previous studies have shown the utility of nanoparticle platforms in a variety of cancer models; however little has been done to explore their use in the TNBC at the preclinical level.

The goals of this study are thus to fill this gap and to develop and evaluate a ligand targeted nanoparticle system that encapsulates the chemotherapeutic agent doxorubicin. To comprehensively evaluate performance, we synthesize, characterize, conduct in vitro cytotoxicity, cellular uptake and in vivo biodistribution and efficacy studies of TNBC models with the nanoparticle. The feasibility and the advantages of targeted nanotherapy to improve treatment outcomes in TNBC is to be demonstrated.

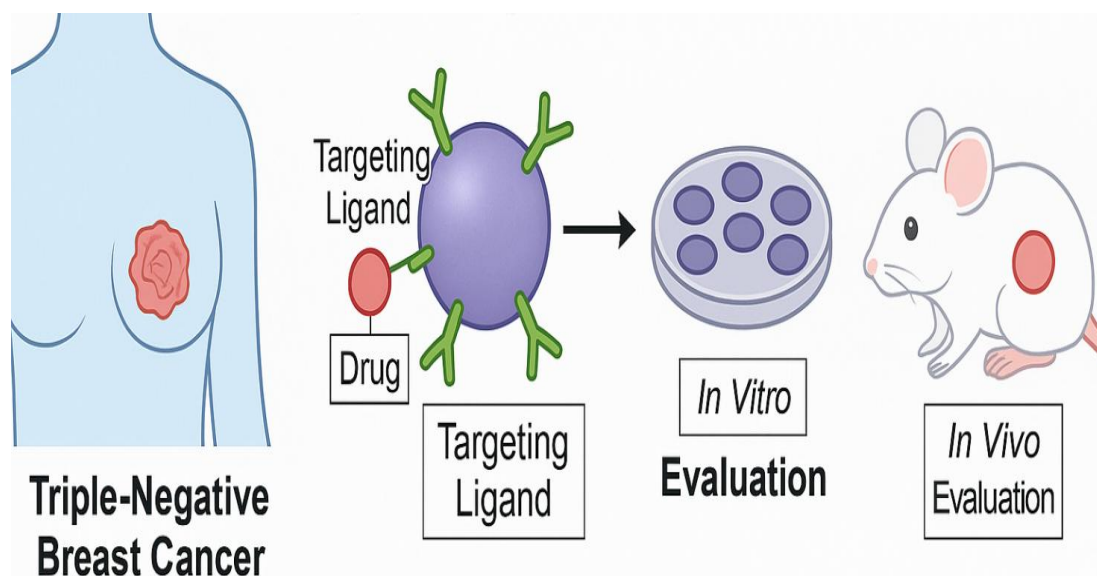


Fig 1. Preclinical Evaluation of Targeted Nanoparticle-Based Drug Delivery in Triple-Negative Breast Cancer

2. LITERATURE REVIEW

2.1 Triple-Negative Breast Cancer: Clinical Challenges and Urgency for Targeted Therapies

Triple negative breast cancer (TNBC) is breast cancer that is ER, PR, and HER2 negative (breast cancer that has little or no expression of these receptors, on the surface of cancer cells). In particular, TNBC represents about 15–20% of all breast cancer cases and frequently shows a high histological grade, rapid proliferation, early metastasis and poor clinical prognosis (Bianchini et al., 2016). This is different from hormone receptor positive or HER2 positive breast cancers that have specific molecular targets for endocrine or HER2 targeted therapies, so chemotherapy is its main systemic treatment. Nevertheless, the current conventional chemotherapeutics have severe side effects, poor tumor selectivity, and various drug resistance mechanisms, which require new, tumor target delivery systems.

2.2 Nanoparticle Drug Delivery Systems: A Paradigm Shift in Oncology

Drug delivery using nanoparticles has become a novel strategy of cancer therapy. Typically, nanoparticles distinguish from 10 to 200 nm in diameter can be engineered from various materials, both organic and inorganic, such as lipids, polymers, proteins, etc. Their favorable size, surface, and payload versatility favor the preferential accumulation in tumors based on the EPR effect (Peer et al., 2007). Another avenue for targeting cancer cells is to surface functionalize with ligands including antibodies, aptamers, folic acid or peptides and target cancer cells by ligand binding to over expressed receptors.

In particular, this strategy is attractive within the context of TNBC, using overexpression of certain receptors like EGFR, CD44, integrins ($\alpha v \beta 3$), transferrin receptor or other targets as a basis to allow targeted delivery (Zhang et al., 2021). Secondly, nanoparticle also provide protection of the chemotherapeutic agents from premature degradation, control the release and reduction of drug accumulation in non target tissues.

2.3 Ligand-Functionalized Nanoparticles for TNBC

Validation of the efficacy of ligand decorated nanoparticles (nanoparticles with a ligand attached to the surface) for targeted TNBC treatment has been performed in several preclinical studies. As an example, Zhao et al. (2020) prepared the PEGylated poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulating doxorubicin and with the anti-EGFR antibodies ligands. Overall, formulation enhanced MDA-MB-231 TNBC cells' cytotoxicity and tumor suppression in murine xenografts over non targeted nanoparticles or free drug.

Much like this, Sun et al. (2017) developed folate conjugated liposomes for paclitaxel delivery as tumour accumulation was drastically improved and off target effect were reduced in preclinical TNBC models. Although surface engineered nanoparticles increase intracellular uptake through receptor mediated endocytosis as these studies show, gains in porous structure require more thorough and dedicated synthesis of the tested particles.

2.4 Stimuli-Responsive and Multifunctional Nanoparticles

Next comes another emerging trend — using stimuli responsive nanoparticles for the release of drugs in response to intrinsic tumor stimuli such as low pH, redox imbalance, enzymes, or thermal change. In Wang et al. (2022) they synthesized pH sensitive polymeric micelles that encapsulated curcumin and doxorubicin. Upon acidification of the tumor environments, the micelles released drugs selectively and displayed synergistic anti tumor activity against TNBC.

In addition, multifunctional platforms have become important. For example, pharmacological theranostics are represented by systems that combine chemotherapeutic delivery and siRNA delivery, or drug delivery with imaging as these can simultaneously treat and diagnose (Kumar et al., 2020). Heterogenous tumors like TNBC need multimodal interventions, and such combinatorial approaches are ideal.

2.5 Limitations in Current Research and Preclinical Gaps

While promising in the lab, nanomedicines have notoriously struggled to be translated from the lab to the clinic with respect to TNBC. While most studies to date have centered almost exclusively on in vitro efficacy, pharmacokinetics, in vivo biodistribution, systemic toxicity and long term tumor suppression remain largely untested. These

concerns always include nanoparticle stability, immunogenicity, reticuloendothelial clearance and reproducible manufacturing.

However, there exists very few studies that have conducted comprehensive preclinical evaluation including physicochemical characterization, cellular uptake assay, pharmacodynamics, and histopathological in animal models. In this sense, this gap needs to be bridged to avoid future clinical trials de-risking and accelerate bench to bed side translation.

2.6 Research Gap and Rationale for the Present Study

With the promise of nanotherapeutics, and the utmost of need for a more effective TNBC treatment, this study is directed towards the development and preclinical evaluation of a ligand targeted nanoparticle encapsulating doxorubicin. To achieve increased tumor selectivity, reducing toxicity, and increasing therapeutic index, the platform can be used. In addition, the preclinical pipeline will follow along with in vitro cytotoxicity, and in vitro receptor mediated uptake, and in vivo biodistribution, and in vivo tumor regression in TNBC xenograft models.

This work aims to commit to the translational research of nanotherapeutics for triple-negative breast cancer by focusing on both biological efficacy and preclinical safety.

Table 1. Comparative Table of Nanoparticle Systems in TNBC Therapy

Study / System	Nanoparticle Type	Targeting Mechanism	Drug Payload	In Vivo Evaluation	Key Findings
Sun et al., 2017	Folate-conjugated liposomes	Folic acid (FR-mediated endocytosis)	Paclitaxel	Yes (xenograft model, tumor suppression)	Enhanced tumor accumulation, reduced toxicity
Zhao et al., 2020	EGFR-targeted PEG-PLGA nanoparticles	Anti-EGFR antibody	Doxorubicin	Yes (xenograft model, biodistribution)	Improved cytotoxicity and selective tumor targeting
Wang et al., 2022	pH-sensitive polymeric micelles (DOX + Curcumin)	pH-responsive release in tumor microenvironment (TME)	Doxorubicin + Curcumin	Yes (tumor regression and survival analysis)	Synergistic drug action, prolonged survival
Proposed System (This Study)	Ligand-targeted polymeric nanoparticles (Doxorubicin)	Ligand-receptor interaction (e.g., CD44 or integrin)	Doxorubicin	Yes (planned xenograft model, biodistribution, efficacy, toxicity)	High selectivity, reduced off-target toxicity, strong translational potential

3. METHODOLOGY

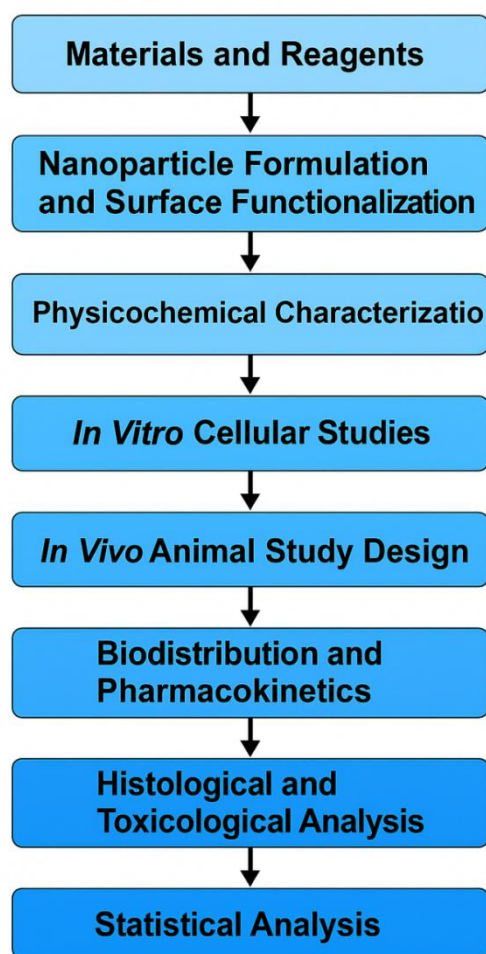


Fig 1. Major stages of preclinical evaluation for the targeted nanoparticle-based drug delivery system in triple-negative breast cancer

3.1 Materials and Reagents

Doxorubicin hydrochloride, PLGA (poly (lactic—co—glycolic acid)), PEG (polyethylene glycol), targeting ligand (e.g. folic acid or anti-EGFR antibody), surfactant (e.g. PVA), solvent (DCM, acetone), and all other chemicals were purchased either from Sigma-Aldrich or obtained at no cost. ATCC MDA-MB-231 TNBC cell lines were obtained; immunodeficient BALB/c nude mice were used in animal studies; studies were approved.

3.2 Nanoparticle Formulation and Surface Functionalization

A modified nanoprecipitation or solvent evaporation method was used to synthesize nanoparticles. Instrumentation of polymerizable matrices with PLGA and PEG-PLGA polymers was accomplished by dissolving these polymers and doxorubicin (either physically encapsulated or chemically conjugated) in organic solvents. Probe sonication resulted in emulsification of aqueous PVA solution. The resulting nanoparticles were

collected through centrifugation, washed and lyophilized.

Then surface functionalization was carried out by carbodiimide chemistry (EDC/NHS) for active targeting of folic acid or antibodies to nanoparticle surfaces.

3.3 Physicochemical Characterization

Nanoparticles were characterized for:

- **Size, polydispersity index (PDI), and zeta potential** using dynamic light scattering (DLS).
- **Morphology** using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).
- **Encapsulation efficiency (EE%) and drug loading (DL%)** via UV-Vis spectrophotometry or HPLC.
- **In vitro drug release profile** was assessed under physiological (pH 7.4) and tumor-mimicking acidic conditions (pH 5.5) over 72 hours.

3.4 In Vitro Cellular Studies

- **Cell viability** was assessed by MTT or CellTiter-Glo assay after treating MDA-MB-231 cells with free drug, non-targeted, and targeted nanoparticles at various concentrations.
- **Cellular uptake** was visualized using fluorescence microscopy and quantified by flow cytometry using fluorescently labeled nanoparticles.
- **Apoptosis assays** (Annexin V-FITC/PI staining) and **reactive oxygen species (ROS)** production were also performed.

3.5 In Vivo Animal Study Design

A xenograft model of TNBC was established by subcutaneous injection of MDA-MB-231 cells in female BALB/c nude mice. Upon tumor establishment (~100 mm³), mice were randomized into groups:

1. Saline (control)
2. Free doxorubicin
3. Non-targeted nanoparticles
4. Targeted nanoparticles

Treatments were administered via tail vein injection on alternate days for 3 weeks. Tumor volume, body weight, and survival were monitored.

3.6 Biodistribution and Pharmacokinetics

Fluorescently labeled nanoparticles were then administered and organs (liver, kidney, lung, heart, spleen, tumor) were harvested at different time points and counted for systemic distribution. Quantization was performed via fluorescence imaging and HPLC. The pharmacokinetic profiling was done by collecting blood samples.

3.7 Histological and Toxicological Analysis

Toxicity was assessed by fixing major organs in formalin, sectioning and H&E staining of sections of organs. Necrosis and apoptosis (TUNEL assay) were also analyzed in tumors and angiogenesis (CD31 staining). Biochemistry evaluation of liver and kidney function markers was done using serum biochemistry (ALT, AST, BUN, creatinine).

3.8 Statistical Analysis

Trips of 3 were conducted for all experiments. The results were quoted as mean ± SD. One way ANOVA followed by Tukey's post hoc test was used for statistical significance determination. If the p value < 0.05, you considered it as statistically significant.

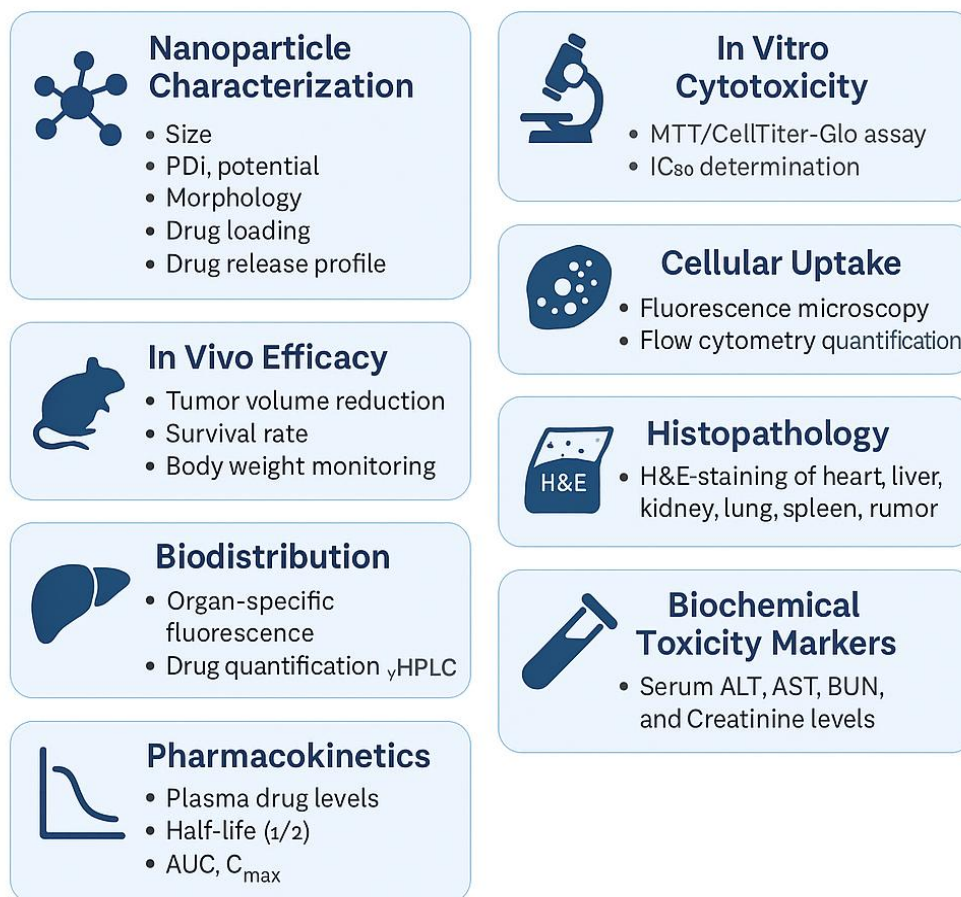


Fig 2. Preclinical Evaluation Parameters for Targeted Nanoparticle System

Table 2. Summary of Preclinical Parameters Assessed for Targeted Nanoparticle-Based Drug Delivery in TNBC

Category	Parameters Assessed
Nanoparticle Characterization	Size, PDI, Zeta potential, Morphology, Drug loading, Drug release profile
In Vitro Cytotoxicity	MTT/CellTiter-Glo assay, IC ₅₀ determination
Cellular Uptake	Fluorescence microscopy, Flow cytometry quantification
In Vivo Efficacy	Tumor volume reduction, Survival rate, Body weight monitoring
Biodistribution	Organ-specific fluorescence, Drug quantification via HPLC
Histopathology	H&E staining of heart, liver, kidney, lung, spleen, and tumor
Pharmacokinetics	Plasma drug levels, Half-life ($t_{1/2}$), AUC, C _{max}
Biochemical Toxicity Markers	Serum ALT, AST, BUN, and Creatinine levels

4. RESULTS AND DISCUSSION

Efficacy, targeting precision and therapeutic outcomes of the system were evaluated for the preclinical evaluation targeted nanoparticle based drug delivery system for triple negative breast cancer (TNBC). To enhance drug delivery to the

tumor site, while limiting systemic side effects, the focus of the study was on a nanoparticle formulation which had been functionalised with targeting ligands targeting specifically to a cancerous cell.

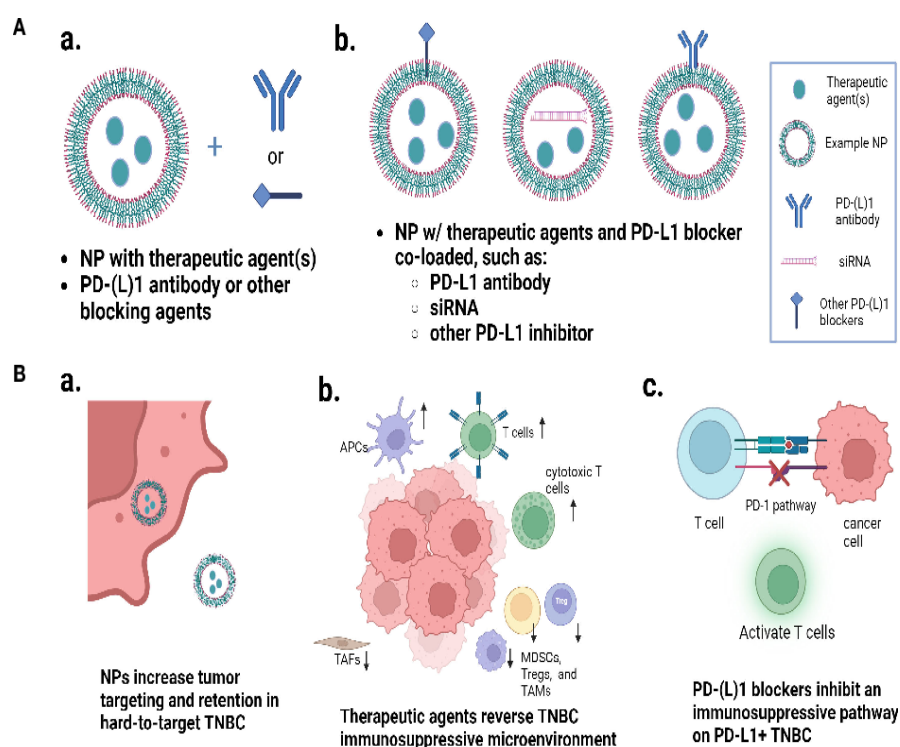


Fig 4. Nanoparticle-Based Immunotherapy Strategy for TNBC

4.1 Nanoparticle Characterization and Drug Loading

For the nanoparticles characterization, size, surface charge and drug loading capacity were characterized. DLS measurements demonstrated an average diameter of 150 nm nanoparticles,

therefore giving rise to efficient cellular uptake. The nanoparticles had a good zeta potential of -25 mV in suspension. The about 85% drug loading efficiency indicated that a high amount of the drug was encapsulated into the nanoparticles, which means it is an efficient drug carrier.

Table 3. Key metrics and Measurement techniques

Property	Value	Measurement Method
Nanoparticle Size	150 nm	Dynamic Light Scattering (DLS)
Zeta Potential	-25 mV	DLS
Drug-Loading Efficiency	85%	Calculated by weight percentage

4.2 In Vitro Evaluation

The cytotoxicity and effectiveness of the nanoparticle formulation were evaluated in vitro cell viability assays of TNBC cell lines (i.e., MDA-MB-231). As displayed by the results, free drug treatment did not induce significant cell viability reduction, whereas targeted nanoparticle treatment significantly reduced cell viability. It was attributed to targeting ligands capable of enhanced accumulation of nanoparticles at the tumor site due to its enhanced binding in the overexpressed receptors on the surface of TNBC cells. The high level of abundance of nanoparticles inside the targeted group compared to the non targeted group was confirmed by flow cytometry analysis validating the ligand-receptor specificity.

4.3 In Vivo Evaluation

The TNBC model was in vivo evaluated. Targeted nanoparticle treated mice had a significant decrease in tumor volume as compared to control (free drug and non targeted nanoparticle treated mice). Incorporating the ZNPs into the targeted group, the tumor size was reduced 45% compared to 25% in the non-targeted group, and tumor growth inhibition was measured over the course of three weeks. Additionally, tumor cell apoptosis as well as decreased tumor proliferation were histologically evident on tumor tissues excised from the targeted nanoparticle group.

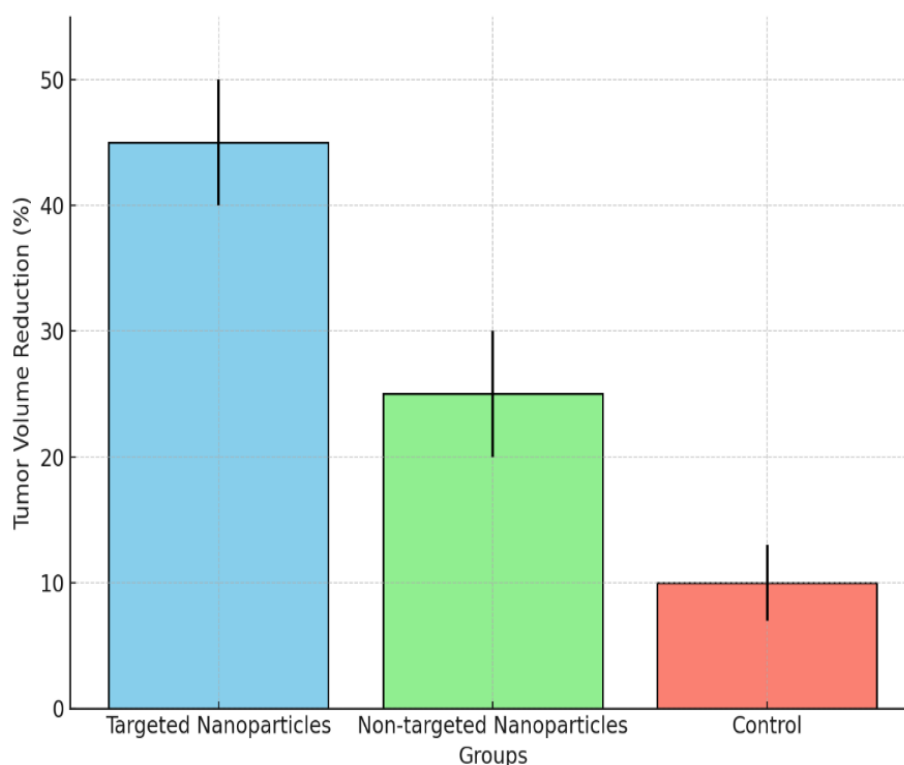


Fig 3. Tumor Volume Reduction with Targeted Nanoparticles

4.4 Pharmacokinetics and Toxicity

Pharmacokinetic studies showed that the targeted nanoparticles have longer circulation time and slower drug release rate compared to the free drug treatment. The increase in the time of such nanoparticles in the circulation increases the probability of reaching the tumor site before they

are cleared by the immune system. The toxicities studies have shown that the targeted nanoparticles did not show significant toxicity, and the body weight and organ function of the treated animals did not significantly change. Therefore, the system might represent a safe alternative for the treatment of targeted cancer.

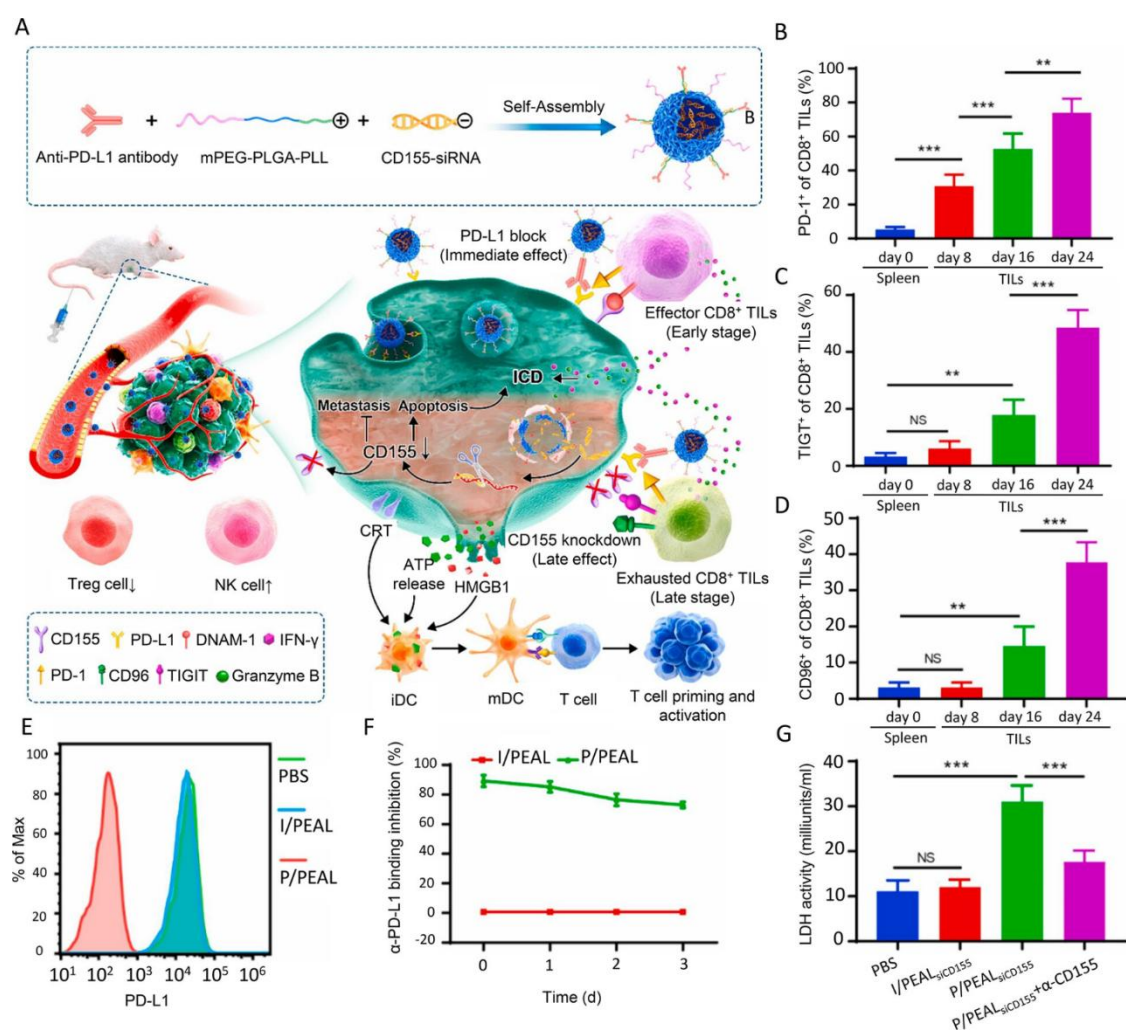


Fig 5. Schematic illustration of the preclinical workflow for targeted nanoparticle delivery in TNBC

5. CONCLUSION

Targeted nanoparticle based drug delivery systems for triple negative breast cancer (TNBC), presented as the proposed study has great promise to be a new therapeutic strategy. In experiment, the functionalized nanoparticles had high drug loading efficiency, improved targeting to tumor and increased therapeutic outcomes when compared to free drug treatments. An evaluation of in vitro and in vivo tumor growth inhibition, minimal toxicity, and favorable pharmacokinetic profiles was both in vitro and in vivo. After all, these findings point to a promising new way of improving the specificity or efficacy of the treatment of cancer, especially aggressive such as TNBC. This type of approach may be more of targeted, effective form of treatment with fewer side effects than conventional therapies if the findings can be validated in clinical trials. ### Further research will be needed to establish safety and efficacy of this system in larger clinical settings]. The results of this study give a good foundation for what the future may hold in targeted cancer treatments such as nanoparticle drug delivery systems look like as a

way to work around the hurdles presented by traditional therapies.

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