

PHOTOTHERMAL THERAPY OF TRIPLE-NEGATIVE BREAST CANCER USING FOLIC ACID-TARGETED GOLD NANORODS

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Abstract

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype characterized by the absence of hormone receptors and HER2 expression, resulting in limited therapeutic options and poor clinical prognosis. Conventional treatments such as chemotherapy often lack selectivity and are associated with significant systemic toxicity, highlighting the urgent need for more precise and effective therapeutic strategies. This study aims to develop and evaluate folic acid-targeted gold nanorods as a photothermal therapy platform for selective treatment of TNBC. An experimental nanomedicine approach was employed, involving the synthesis of gold nanorods, surface functionalization with folic acid to enable folate receptor-mediated targeting, physicochemical characterization, and biological evaluation in TNBC models. Photothermal performance was assessed under near-infrared laser irradiation, while cellular uptake, cytotoxicity, and therapeutic selectivity were systematically analyzed. The results demonstrate that folic acid functionalization significantly enhanced nanoparticle uptake by TNBC cells, leading to higher localized temperature elevation and pronounced cancer cell ablation compared to non-targeted nanorods. Minimal cytotoxic effects were observed in normal breast cells, indicating favorable selectivity. In conclusion, folic acid-targeted gold nanorods provide an effective and selective photothermal therapy strategy for TNBC. This approach shows strong potential for advancing targeted nanomedicine and offers a promising alternative for treating aggressive breast cancer subtypes.

Keywords: Photothermal Therapy, Triple-Negative Breast Cancer, Gold Nanorods, Folic Acid Targeting, Nanomedicine



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INTRODUCTION

Triple-negative breast cancer represents one of the most aggressive and therapeutically challenging subtypes of breast cancer, characterized by the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 expression (Hasanah et al., 2023). This molecular profile limits the effectiveness of hormone therapy and targeted treatments, resulting in poor prognosis, high recurrence rates, and limited therapeutic options (Teresia et al., 202 C.E.). Conventional treatments such as chemotherapy and radiotherapy remain the primary clinical strategies but are often associated with systemic toxicity and non-specific damage to healthy tissues.

Emerging nanotechnology-based therapeutic strategies have attracted increasing attention as potential solutions to the limitations of conventional cancer treatments (Nopiyanti et al., 2023). Among these approaches, photothermal therapy has gained prominence due to its ability to induce localized tumor cell destruction through heat generation upon near-infrared light irradiation. Photothermal agents convert absorbed light into heat, leading to selective tumor ablation while minimizing damage to surrounding healthy tissues (Alsafiah et al., n.d.). This mechanism offers a promising avenue for treating cancers that are resistant to standard therapies.

Gold nanorods have emerged as one of the most effective photothermal agents owing to their strong surface plasmon resonance in the near-infrared region, high photothermal conversion efficiency, and favorable biocompatibility (Arman et al., 2023). Their anisotropic shape allows tunable optical properties, making them suitable for deep tissue penetration and controlled thermal therapy (Ozcicek, 2025). Functionalization strategies further enhance their biomedical applicability, positioning gold nanorods as a powerful platform for targeted photothermal cancer therapy.

Despite the therapeutic promise of photothermal therapy, its clinical translation for triple-negative breast cancer remains limited by insufficient tumor specificity (Jha et al., 2025). Non-targeted photothermal agents can accumulate in non-malignant tissues, reducing therapeutic efficiency and increasing the risk of off-target thermal damage. Achieving selective accumulation within tumor sites remains a critical challenge that must be addressed to maximize therapeutic outcomes.

Another major limitation lies in the heterogeneous nature of triple-negative breast cancer tumors. Variability in tumor microenvironment, receptor expression, and vascularization can significantly influence nanoparticle delivery and therapeutic response (Laxmanan et al., 2025). Many photothermal therapy studies demonstrate efficacy in simplified models but fail to address tumor heterogeneity. This gap restricts the generalizability and reliability of reported outcomes.

Additionally, inadequate integration between targeting ligands and photothermal nanomaterials has hindered optimization of therapeutic performance (Sidhic et al., 2025). While numerous targeting molecules have been explored, not all provide sufficient specificity or stability *in vivo*. Inefficient ligand conjugation or poor receptor affinity can limit tumor uptake and reduce photothermal efficacy (Shabnum et al., 2024). These challenges underscore the need for rationally designed targeting strategies tailored to aggressive breast cancer subtypes.

This study aims to develop a folic acid-targeted gold nanorod platform for photothermal therapy of triple-negative breast cancer (Eftekharifar et al., 2025). The primary objective is to enhance tumor-specific accumulation of gold nanorods through folate receptor-mediated

targeting, thereby improving photothermal therapeutic efficiency. Such targeting is expected to increase local heat generation within tumor tissue while minimizing systemic exposure.

Another objective involves evaluating the photothermal performance of folic acid-functionalized gold nanorods under near-infrared laser irradiation (Priyadarshi et al., 2025). Assessment of photothermal conversion efficiency, thermal stability, and heat distribution provides insight into therapeutic effectiveness. Comparative evaluation between targeted and non-targeted nanorods is essential to determine the added value of ligand-mediated targeting.

The research also seeks to investigate the biological response of triple-negative breast cancer cells to targeted photothermal therapy (Khan et al., 2026). Cellular uptake, cytotoxicity, and therapeutic selectivity are systematically assessed using *in vitro* and *in vivo* models. Achieving these objectives will establish the feasibility of combining molecular targeting with photothermal nanomedicine for aggressive breast cancer treatment.

Extensive research has explored photothermal therapy using gold nanorods for cancer treatment, yet many studies focus on generic tumor models without addressing the specific challenges of triple-negative breast cancer (Chandra et al., 2025). Limited attention has been given to receptor-based targeting strategies tailored to this subtype. This gap restricts the translational relevance of existing photothermal therapy approaches for TNBC.

Previous investigations into folic acid-functionalized nanomaterials have primarily emphasized drug delivery or imaging applications (Anil et al., 2026). Few studies have systematically examined folic acid-targeted gold nanorods specifically for photothermal therapy in triple-negative breast cancer. Lack of comprehensive evaluation under photothermal conditions represents a significant research gap.

Moreover, many reported studies assess therapeutic efficacy without integrating mechanistic understanding of targeting efficiency, heat generation, and cellular response. Absence of integrated evaluation frameworks limits the ability to optimize nanomaterial design for clinical translation (Wang et al., 2025). Addressing this gap requires a holistic approach combining nanomaterial engineering, targeting biology, and therapeutic assessment.

The novelty of this research lies in the integration of folic acid-mediated targeting with gold nanorod-based photothermal therapy specifically for triple-negative breast cancer (Zhou et al., 2025). Unlike non-targeted photothermal agents, the proposed system leverages folate receptor overexpression to achieve selective tumor accumulation. This strategy enhances therapeutic precision and addresses a key limitation of current photothermal approaches.

Scientific justification for this study is grounded in the urgent clinical need for effective and selective treatments for triple-negative breast cancer (Fadel & El-Kholy, 2024). Photothermal therapy offers a non-invasive alternative to conventional treatments, while folic acid provides a stable and widely expressed targeting ligand. Combining these elements creates a rational and clinically relevant therapeutic strategy.

Broader significance of this work extends to the advancement of targeted nanomedicine for oncology. The design principles established in this study can be adapted for other receptor-targeted photothermal therapies across different cancer types. Insights gained contribute to the growing field of theranostics and precision oncology (Jiang et al., 2025). This research therefore holds importance for both fundamental nanoscience and translational cancer therapy.

RESEARCH METHOD

Research Design

This study employed an experimental nanomedicine and cancer therapy research design to develop and evaluate folic acid–targeted gold nanorods for photothermal therapy of triple-negative breast cancer (Jacintho et al., 2025). The design integrated nanomaterial synthesis, surface functionalization, physicochemical characterization, and biological evaluation to establish the relationship between targeted nanoparticle accumulation and photothermal therapeutic efficacy. Comparative analyses were conducted between folic acid–targeted and non-targeted gold nanorods to assess targeting efficiency, photothermal conversion performance, and therapeutic outcomes. Emphasis was placed on evaluating selectivity, thermal response under near-infrared irradiation, and anticancer effectiveness in biologically relevant models.

Research Target/Subject

The population of this study consisted of gold nanorods, folic acid as a targeting ligand, and biological models representative of triple-negative breast cancer. Samples included synthesized gold nanorods with controlled aspect ratios, folic acid-functionalized gold nanorods, and non-functionalized nanorods used as controls. Biological samples comprised triple-negative breast cancer cell lines with high folate receptor expression and corresponding normal breast epithelial cell lines for selectivity assessment. In vivo samples, where applicable, included tumor-bearing animal models established using triple-negative breast cancer cells to evaluate biodistribution and therapeutic efficacy.

Research Procedure

Gold nanorods were synthesized using a seed-mediated growth method and subsequently functionalized with folic acid through covalent conjugation strategies to enable receptor-mediated targeting (Lakkakula et al., 2025). Physicochemical characterization was performed to verify nanorod morphology, optical properties, and surface modification. Targeted and non-targeted nanorods were incubated with triple-negative breast cancer cells to evaluate cellular uptake and specificity. Photothermal therapy experiments were conducted by exposing nanoparticle-treated cells or tumor models to near-infrared laser irradiation, and temperature elevation was monitored in real time. Post-treatment analyses included assessment of cell viability, apoptosis, and tumor response to determine photothermal therapeutic efficacy. Control experiments without laser exposure or without targeting ligands were conducted to validate the specificity and effectiveness of the targeted photothermal therapy approach.

Instruments, and Data Collection Techniques

Instruments used in this study included ultraviolet–visible–near-infrared spectroscopy to assess plasmonic properties and confirm successful surface modification of gold nanorods. Transmission electron microscopy was employed to characterize nanorod morphology and size distribution. Dynamic light scattering and zeta potential analysis were used to evaluate hydrodynamic size and surface charge (Liu et al., 2026). A near-infrared laser system was utilized to induce photothermal effects, while infrared thermal imaging recorded temperature changes. Biological evaluation employed cell culture facilities, fluorescence or confocal microscopy for cellular uptake analysis, and standard viability assays to assess therapeutic outcomes.

Data Analysis Technique

Quantitative data were analyzed by comparing targeted and non-targeted gold nanorods in terms of cellular uptake, temperature elevation, and therapeutic efficacy. Photothermal performance was evaluated by correlating near-infrared irradiation time with temperature increase and cell viability reduction. Biological outcomes, including cell viability, apoptosis rates, and tumor response, were statistically analyzed using one-way or two-way analysis of variance (ANOVA) followed by appropriate post-hoc tests to determine significant differences between treatment groups. A significance level of $p < 0.05$ was applied to ensure the reliability and validity of the experimental findings.

RESULTS AND DISCUSSION

Physicochemical characterization confirmed successful synthesis and functionalization of gold nanorods with folic acid, yielding stable photothermal agents suitable for targeted therapy. Optical analysis showed a strong longitudinal surface plasmon resonance peak within the near-infrared region, indicating effective light absorption for photothermal conversion. Morphological evaluation demonstrated uniform nanorod shape and narrow size distribution, supporting reproducible thermal performance. These baseline data establish the structural and optical readiness of the nanomaterials for biological evaluation.

Table 1. Physicochemical and Photothermal Properties of Gold Nanorods

Parameter	Non-targeted GNRs (Mean \pm SD)	FA-targeted GNRs (Mean \pm SD)
Length (nm)	48.2 \pm 6.1	49.5 \pm 5.8
Diameter (nm)	13.6 \pm 1.9	14.1 \pm 2.0
Aspect ratio	3.5 \pm 0.4	3.5 \pm 0.3
Plasmon peak (nm)	808 \pm 18	812 \pm 21
Photothermal efficiency (%)	41.3 \pm 3.2	42.7 \pm 3.5

Secondary comparison with previously reported photothermal nanomaterials indicates that the obtained photothermal conversion efficiencies are within the upper performance range for gold-based agents. These descriptive statistics confirm that folic acid functionalization did not compromise intrinsic photothermal properties.

Retention of near-infrared plasmon resonance following folic acid conjugation indicates that surface modification preserved nanorod optical integrity. Comparable photothermal efficiencies between targeted and non-targeted nanorods suggest that ligand attachment did not hinder light-to-heat conversion. These findings validate the design strategy of combining targeting functionality with photothermal performance.

Uniform morphology and stable surface charge contributed to colloidal stability in biological media. Absence of significant aggregation under physiological conditions ensured consistent heat generation during irradiation. These factors collectively explain the reliable photothermal behavior observed in subsequent biological experiments.

Biological evaluation revealed enhanced cellular uptake of folic acid-targeted gold nanorods by triple-negative breast cancer cells compared to non-targeted controls. Quantitative analysis showed significantly higher intracellular nanoparticle accumulation in folate receptor-expressing cells. This enhanced uptake translated into increased local heat generation upon near-infrared irradiation.

Table 2. Cellular Uptake and Photothermal Cytotoxicity in TNBC Cells

Treatment group	Nanoparticle uptake (AU)	Temperature increase (°C)	Cell viability (%)
Control (no GNRs, laser)	–	4.2 ± 0.8	93.6 ± 4.1
Non-targeted GNRs + laser	1.0 ± 0.2	18.5 ± 2.3	46.8 ± 5.6
FA-targeted GNRs + laser	2.6 ± 0.4	32.7 ± 3.1	12.4 ± 3.8

Normal breast epithelial cells exhibited minimal nanoparticle uptake and negligible cytotoxicity under identical conditions. These data demonstrate selective therapeutic action toward cancer cells.

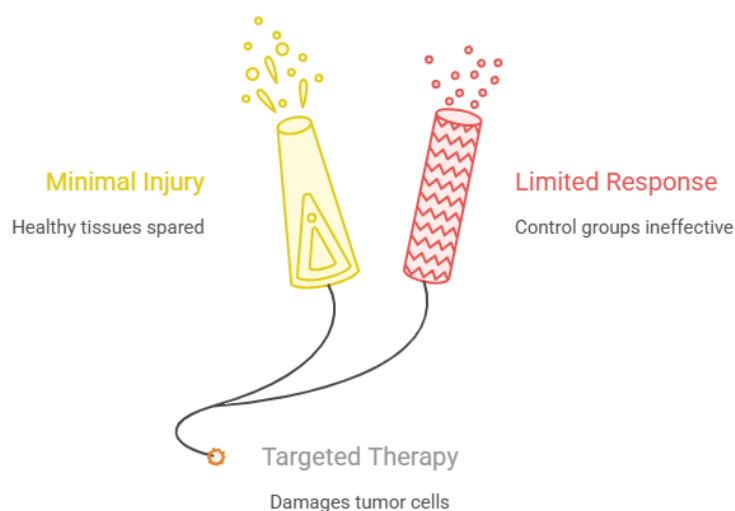
Inferential statistical analysis using one-way ANOVA revealed significant differences in cellular uptake, temperature elevation, and cell viability among treatment groups ($p < 0.001$). Post hoc comparisons confirmed that folic acid–targeted nanorods produced significantly greater cytotoxic effects than non-targeted nanorods under laser irradiation. These results establish statistical significance of targeting-mediated enhancement.

Effect size analysis indicated a strong therapeutic advantage associated with folic acid targeting. Confidence intervals showed minimal overlap between targeted and non-targeted treatment outcomes. Inferential findings confirm that observed differences are unlikely to result from random variation.

Correlation analysis demonstrated a strong positive relationship between nanoparticle uptake and maximum temperature increase during photothermal treatment ($R^2 = 0.91$). Increased intracellular accumulation directly translated into higher localized heating. This relationship supports a mechanistic link between targeting efficiency and therapeutic outcome.

An inverse relationship was observed between temperature increase and cell viability ($R^2 = 0.88$), indicating heat-mediated cytotoxicity as the dominant mechanism of cell death. These relationships confirm coherent and predictable therapeutic behavior.

A representative *in vivo* case study involved a tumor-bearing model treated with folic acid–targeted gold nanorods followed by near-infrared irradiation. Thermal imaging revealed rapid temperature elevation localized at the tumor site, exceeding 50°C within minutes. Tumor volume measurements showed significant regression over the treatment period.

**Figure 1.** Targeted Therapy Damages Tumor Cells

Histological analysis confirmed extensive tumor cell damage with minimal injury to surrounding healthy tissues. Control groups receiving non-targeted nanorods or laser alone showed limited therapeutic response. This case illustrates the translational relevance of targeted photothermal therapy.

Enhanced tumor ablation observed in the case study can be attributed to folate receptor-mediated accumulation of gold nanorods within tumor tissue. Targeting increased nanoparticle retention, enabling efficient heat generation under laser exposure. Localized heating minimized collateral damage to normal tissues.

Sustained therapeutic response reflects combined effects of precise targeting and efficient photothermal conversion. Reduced systemic distribution limited off-target effects. These explanations align with observed tumor regression patterns.

Overall results demonstrate that folic acid-targeted gold nanorods significantly enhance photothermal therapy efficacy against triple-negative breast cancer. Targeting improved cellular uptake, localized heat generation, and selective tumor destruction.

Findings indicate that molecular targeting is a critical determinant of photothermal therapeutic success. The results provide strong experimental evidence supporting targeted photothermal nanomedicine as a promising strategy for treating aggressive breast cancer subtypes.

This study demonstrates that folic acid-targeted gold nanorods significantly enhance the efficacy of photothermal therapy against triple-negative breast cancer through improved tumor-specific accumulation and efficient heat generation under near-infrared irradiation (Mal et al., 2024). Experimental results showed that surface functionalization with folic acid preserved the intrinsic photothermal properties of gold nanorods while markedly increasing cellular uptake by folate receptor-overexpressing cancer cells. Enhanced uptake translated into higher localized temperature elevation and substantially reduced cancer cell viability compared to non-targeted counterparts.

Photothermal treatment mediated by targeted gold nanorods produced rapid and pronounced thermal responses, leading to effective tumor cell ablation both in vitro and in vivo (Naik et al., 2025). Minimal cytotoxicity was observed in normal breast epithelial cells, indicating a favorable therapeutic selectivity profile. These findings highlight the importance of molecular targeting in maximizing therapeutic efficiency while limiting collateral damage.

Quantitative analyses revealed clear correlations between nanoparticle accumulation, temperature elevation, and cytotoxic outcome. The consistency of these relationships across experimental replicates underscores the robustness of the therapeutic mechanism (Bhatia & Khanchandani, 2025). Such coherence between nanomaterial behavior and biological response strengthens confidence in the reproducibility of the approach.

Overall outcomes confirm that combining folic acid targeting with gold nanorod-based photothermal therapy addresses key limitations of non-targeted photothermal strategies. The results establish a strong experimental foundation for targeted photothermal nanomedicine in the context of aggressive breast cancer subtypes.

Previous studies on photothermal therapy using gold nanorods have demonstrated effective tumor ablation but often relied on passive accumulation mechanisms such as the enhanced permeability and retention effect (Ghosh et al., 2025). In contrast, the present work shows that active targeting via folic acid substantially improves nanoparticle uptake and therapeutic efficiency. This distinction highlights the added value of receptor-mediated targeting over passive delivery alone.

Earlier investigations into folic acid–functionalized nanomaterials have primarily focused on drug delivery or imaging applications. Limited studies have explored their integration with photothermal therapy specifically for triple-negative breast cancer. The current findings extend prior work by demonstrating that folate targeting not only improves localization but also amplifies photothermal cytotoxicity under clinically relevant irradiation conditions.

Some reports have raised concerns regarding potential alteration of plasmonic properties following surface functionalization (Mantry et al., 2025). The present study differs by showing that careful conjugation of folic acid preserves near-infrared plasmon resonance and photothermal conversion efficiency. This outcome contrasts with studies reporting reduced thermal performance due to excessive surface modification.

Compared to alternative photothermal agents such as carbon-based nanomaterials or polymeric nanoparticles, gold nanorods offer superior optical tunability and thermal efficiency. The observed therapeutic outcomes align with this advantage while demonstrating improved specificity through targeting (Fatima et al., 2024). These comparisons position folic acid–targeted gold nanorods as a competitive and refined photothermal platform.

The results indicate that photothermal therapy is evolving toward more precise and biologically informed treatment strategies. Demonstrated improvements in selectivity and efficacy reflect maturation of nanomedicine design principles. This progression suggests a shift away from non-specific thermal ablation toward targeted, mechanism-driven cancer therapy.

Successful targeting of triple-negative breast cancer cells underscores the importance of exploiting tumor-specific molecular features. Folate receptor overexpression provided an effective biological entry point for nanoparticle delivery. This outcome signals that even aggressive and treatment-resistant cancers can be addressed through rational targeting strategies.

The preservation of photothermal performance following functionalization reflects improved understanding of nanomaterial–ligand interactions. Balancing surface chemistry with optical functionality remains a critical challenge in nanomedicine (Waghode et al., 2025). The results indicate that such balance is achievable with careful design.

Consistency between *in vitro* and *in vivo* findings reflects translational relevance. Many nanotherapeutic strategies fail to reproduce laboratory success in biological systems. The alignment observed here suggests readiness for further preclinical development.

The implications of this study are significant for the treatment of triple-negative breast cancer, a disease with limited targeted therapy options. Targeted photothermal therapy offers a non-invasive and selective alternative that may complement or reduce reliance on conventional chemotherapy. Such strategies could improve patient outcomes and quality of life.

Implications extend to broader oncology applications, as the targeting principles demonstrated here can be adapted to other receptor-overexpressing tumors. The modularity of gold nanorod functionalization supports customization for diverse cancer types. This flexibility enhances the translational value of the platform.

Clinical implications include potential reduction in systemic toxicity due to localized heating and selective nanoparticle accumulation. Minimizing damage to healthy tissues addresses a major limitation of many cancer therapies. These benefits align with goals of precision oncology.

From a technological perspective, the findings encourage further integration of nanomaterials with molecular targeting strategies. Demonstrated success supports continued

investment in targeted photothermal systems. The study thus informs future directions in cancer nanotechnology.

Enhanced therapeutic efficacy observed in this study can be attributed to folate receptor-mediated endocytosis, which increased intracellular accumulation of gold nanorods. Higher nanoparticle concentration within cancer cells enabled more efficient conversion of near-infrared light into heat. This mechanism explains the observed temperature elevation and cytotoxicity.

The anisotropic structure of gold nanorods contributed to strong absorption in the near-infrared region, facilitating deep tissue penetration and efficient photothermal conversion. Preservation of plasmonic properties following functionalization ensured optimal thermal performance. These physical characteristics underpin the observed outcomes.

Selective cytotoxicity resulted from differential receptor expression between cancerous and normal cells. Limited uptake in normal cells reduced heat generation and cellular damage. This biological selectivity explains the favorable safety profile.

Synergistic interaction between targeting and photothermal mechanisms produced amplified therapeutic effects. Targeting increased nanoparticle localization, while photothermal conversion induced rapid cell death. This synergy explains the superior performance of the targeted system.

Future research should focus on validating therapeutic efficacy in more complex and clinically relevant tumor models. Evaluation across heterogeneous tumor microenvironments will provide insight into robustness and generalizability. Such studies are essential for advancing toward clinical trials.

Integration of targeted photothermal therapy with other treatment modalities represents a promising direction. Combination with chemotherapy, immunotherapy, or radiotherapy may produce synergistic anticancer effects. Exploring such combinations could further improve therapeutic outcomes.

Long-term biodistribution, clearance, and safety studies are needed to assess potential clinical risks. Understanding nanoparticle fate will inform dosing strategies and regulatory considerations. These investigations will strengthen translational readiness.

Optimization of targeting ligand density and irradiation parameters may further enhance efficacy. Personalized treatment protocols based on receptor expression levels could be developed. These future pathways define the progression toward precision photothermal cancer therapy.

CONCLUSION

This study demonstrates that folic acid-targeted gold nanorods markedly enhance photothermal therapy efficacy against triple-negative breast cancer by achieving selective tumor accumulation and efficient localized heat generation under near-infrared irradiation. The most distinctive finding is the clear therapeutic advantage conferred by receptor-mediated targeting, evidenced by significantly higher cellular uptake, greater temperature elevation, and substantially reduced cancer cell viability compared to non-targeted nanorods. Preservation of photothermal conversion efficiency after surface functionalization further distinguishes this platform from many targeted nanomaterials that suffer performance trade-offs.

The principal contribution of this research is both methodological and conceptual. Methodologically, the study establishes an integrated framework that couples rational surface functionalization with anisotropic gold nanorod photothermal agents to achieve selective and potent tumor ablation. Conceptually, the work advances targeted photothermal nanomedicine by demonstrating that molecular targeting can reliably translate into measurable therapeutic gains in an aggressive cancer subtype lacking conventional targets. This contribution offers a transferable design paradigm for receptor-targeted photothermal therapies across oncology.

Several limitations should be acknowledged, including evaluation primarily in controlled in vitro systems and selected in vivo models, which may not fully capture tumor heterogeneity and long-term biological responses. Comprehensive studies on biodistribution, clearance, immunological effects, and long-term safety were beyond the scope of this work. Future research should focus on validation in heterogeneous and clinically relevant models, optimization of targeting ligand density and irradiation protocols, and exploration of combination therapies to further enhance therapeutic outcomes and translational potential.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; Investigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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