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Allosteric ligand-driven smart nanoconjugates for mutation-selective EGFR targeting: A precision approach to overcoming tyrosine kinase inhibitor resistance

Dilpreet Singh[1*], Akshay Kumar[1], Vir Vikram Sharma[1]

[1]School of Pharmaceutical Sciences, CT University, Ferozepur Rd, Sidhwan Khurd, Punjab 142024, India

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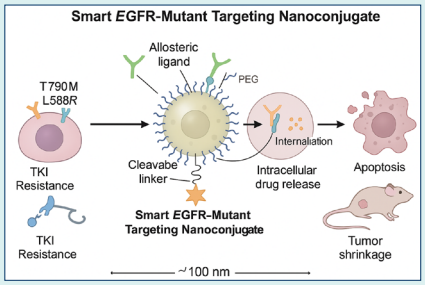
Abstract



The development of targeted therapies against epidermal growth factor receptor (EGFR) has transformed the clinical management of EGFR-driven malignancies, especially non-small cell lung cancer (NSCLC). However, the therapeutic benefit of ATP-competitive tyrosine kinase inhibitors (TKIs) is often undermined by acquired resistance mutations such as T790M and C797S, which either enhance ATP affinity or preclude covalent drug binding. Allosteric inhibition of EGFR has emerged as a promising alternative, leveraging cryptic, mutation-specific binding pockets to achieve superior selectivity and reduced off-target toxicity. Allosteric ligands, particularly those targeting the αC-helix adjacent clefts, have shown potent activity against drug-resistant EGFR isoforms but suffer from suboptimal pharmacokinetics and systemic stability. To overcome these limitations, smart nanoconjugates functionalized with allosteric inhibitors have been developed to enhance targeted delivery, improve intracellular trafficking, and facilitate stimuli-responsive drug release. These nanosystems are capable of co-delivering synergistic agents such as siRNA or CRISPR-Cas9 payloads, amplifying pathway suppression and delaying resistance onset. Surface modification strategies, including PEGylation and bioorthogonal ligand conjugation, further improve circulation half-life and tumor accumulation via active and passive targeting. This review systematically discusses the molecular basis of EGFR allosteric inhibition, engineering principles of nanocarrier platforms, including immunogenicity, scale-up feasibility, and regulatory complexities.

Introduction

Malignant neoplasms, or cancers, remain a leading cause of morbidity and mortality worldwide, with lung, breast, colorectal, and liver cancers accounting for a significant proportion of the global cancer burden. Despite substantial advancements in chemotherapy, targeted therapy, and immunotherapy, many solid tumors exhibit therapeutic resistance, dose-limiting toxicities, and molecular heterogeneity that hinder long-term disease control. Particularly in oncogene-driven malignancies such as non-small cell lung cancer (NSCLC), therapeutic responses are often transient due to acquired mutations, bypass signaling, or clonal evolution.[1,2] The development of molecularly precise therapies that can selectively target aberrant signaling nodes without affecting normal tissues is therefore a critical unmet need in oncology. Against this backdrop, the epidermal growth factor receptor (EGFR) has emerged as a clinically validated but therapeutically challenging target, owing to its dynamic mutational landscape and tendency to develop resistance to ATP-competitive inhibitors.[3] This review highlights the emerging strategy of using allosteric ligand-functionalized smart nanoconjugates for mutation-selective EGFR targeting, offering an integrated solution to overcome resistance, enhance tumor selectivity, and enable combinatorial therapeutic delivery. Over the past two decades, EGFR has emerged as a prototypical molecular target, and the advent of ATP-competitive tyrosine kinase inhibitors (TKIs)—such as gefitinib, erlotinib, afatinib, and osimertinib—has substantially improved patient outcomes.[4] Nevertheless, clinical efficacy is frequently transient due to the rapid development of resistance

*Corresponding author: Dilpreet Singh, Email: dilpreet.daman@gmail.com

mutations such as T790M, C797S, and L718Q, which confer either steric hindrance, increased ATP affinity, or loss of covalent binding potential.[5] Allosteric inhibitors target non-canonical binding sites that emerge due to conformational changes in mutant EGFR isoforms, enabling isoform-specific inhibition while sparing wild-type receptors while sparing wild-type receptors—thus minimizing dose-limiting toxicities and enhancing therapeutic precision.[6] Recent advances in structural biology and molecular dynamics have enabled rational design of allosteric inhibitors with high binding affinity and configurational adaptability. However, the intrinsic instability, poor bioavailability, and systemic clearance of small-molecule allosteric agents necessitate the development of optimized delivery systems.[6,7]

To address these challenges, the field has increasingly converged on *smart nanoconjugates*—nanoscale drug delivery platforms that integrate chemical targeting ligands, responsive release mechanisms, and multivalent architectures to enhance tumor specificity and intracellular delivery.[8] Among these, allosteric ligand-functionalized nanocarriers represent a particularly promising innovation, capable of both passive tumor accumulation via the EPR effect and active engagement of mutant EGFR receptors through high-affinity interactions.[8,9] First, these nanocarriers vehicles protect fragile allosteric ligands from enzymatic degradation and rapid renal clearance, thereby extending systemic half-life. Second, nanocarriers enable preferential accumulation in tumor tissues through the enhanced EPR effect, while functional surface ligands can facilitate active recognition of mutant EGFR isoforms. Hence, the current investigation is situated at this critical interface of molecular pharmacology and nanotechnology.[10] It aims to synthesize recent developments in allosteric EGFR inhibition and highlight how functionalized nanoconjugates offer a precision strategy to overcome drug resistance.[11,12,13] This review consolidates the mechanistic rationale, design principles, and translational challenges of allosteric ligand-driven nanomedicine and proposes a framework for next-generation EGFR-targeted therapies in resistant NSCLC.

EGFR mutational landscape and therapeutic resistance

The mutational landscape of EGFR is central to the pathogenesis, progression, and therapeutic responsiveness of multiple cancers, particularly NSCLC, where EGFR mutations are prevalent in up to 50% of East Asian and 15% of Western patients with adenocarcinoma.[12] The mutational landscape of EGFR and the structural basis of resistance are summarized in Fig. 1, which depicts the spatial positioning of key mutations (L858R, T790M, C797S) and their effects on ATP binding and conformational dynamics. The most common activating mutations—exon 19 deletions and the L858R point mutation in exon 21—lead to constitutive kinase activation, making EGFR an ideal target for first-generation reversible TKIs such as gefitinib and erlotinib.[12,13] However, the clinical efficacy of these agents is transient due to the inevitable emergence of secondary resistance mutations, the most notorious being T790M in exon 20, which increases ATP affinity and sterically hinders inhibitor binding.[12] Third-generation TKIs like osimertinib were developed to selectively inhibit T790M mutants while sparing wild-type EGFR, yet resistance even to these agents has been documented, often via C797S mutation that abrogates covalent binding.[14,15] Additionally, bypass signaling through MET amplification, HER2 overexpression, PIK3CA activation, and phenotypic transformation into small cell lung cancer or epithelial–mesenchymal transition (EMT) contribute to therapeutic escape.[15] These complexities underscore the necessity for mutation-selective therapeutic approaches that can adapt to the heterogeneity and plasticity of EGFR-driven cancers.[16] Recent studies have highlighted the potential of allosteric inhibitors and bi-specific molecules that target mutant-specific conformational states without affecting wild-type EGFR, offering a safer

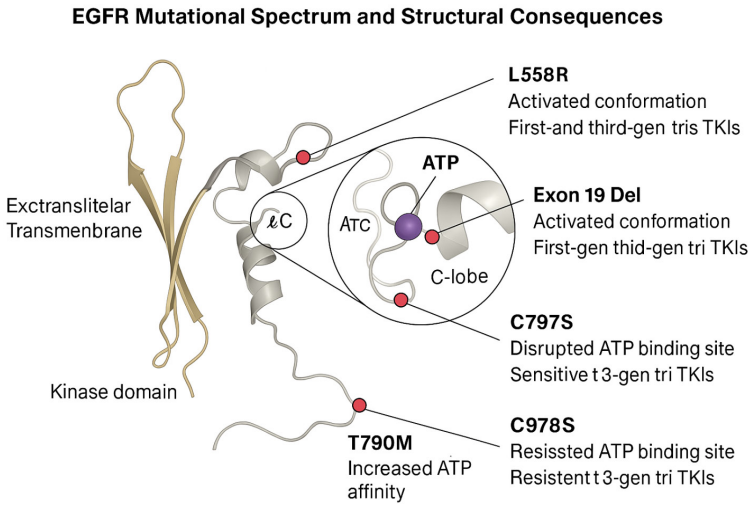


Fig. 1. Structural mapping of key EGFR mutations (L858R, T790M, C797S) and their influence on kinase activity, ATP binding, and resistance mechanisms.

and more durable strategy. Moreover, the integration of next-generation sequencing (NGS) for real-time mutation profiling has facilitated the development of dynamic treatment algorithms and molecularly guided drug delivery systems.[17] These insights emphasize the importance of designing smart nanoconjugates that are functionally responsive to specific EGFR mutation signatures and capable of circumventing multi-level resistance mechanisms, laying the groundwork for more personalized and adaptive targeted therapies. The clinical relevance of specific EGFR mutations—such as L858R, T790M, and C797S—lies in their structural impact on kinase function and their differential sensitivity to various generations of TKIs (Table 1).

Allosteric modulation of EGFR: Mechanistic insights

The concept of allosteric modulation in the context of EGFR (epidermal growth factor receptor) therapy has gained significant traction as a means to overcome resistance mechanisms associated with ATP-competitive TKIs. Allosteric inhibitors bind to regions of the EGFR kinase domain that are topographically distinct from the ATP-binding orthosteric pocket, inducing conformational alterations that impede kinase activity without directly competing for ATP.[18] A mechanistic comparison of allosteric inhibitors versus ATP-competitive TKIs is illustrated in Fig. 2, highlighting how allosteric agents bind outside the catalytic pocket to stabilize the inactive conformation in mutant receptors. Structural studies using X-ray crystallography and cryo-EM have elucidated key allosteric sites, notably near the αC-helix and activation loop (A-loop), which serve as anchor points for small molecules capable of stabilizing inactive kinase conformations.[19] The landmark discovery of EAI045, a mutant-selective allosteric EGFR inhibitor, demonstrated selective efficacy against the T790M and L858R mutations while sparing wild-type EGFR, suggesting a paradigm shift toward safer and mutation-specific therapeutics.[20] Several allosteric EGFR inhibitors, including EAI045 and JBJ-04-125-02, have demonstrated nanomolar potency against drug-resistant EGFR isoforms while exhibiting minimal activity against wild-type EGFR (Table 2). Their structural diversity and non-ATP competitive binding profiles make them ideal candidates for conjugation to nanosystems.[21] These inhibitors often exhibit synergism with ATP-site binders due to non-overlapping binding modalities, effectively locking the kinase in a catalytically inactive state. Moreover, allosteric binding tends to be less affected by mutations that elevate ATP affinity, such as T790M, making this approach particularly suitable for drug-resistant NSCLC.[22] Recent molecular dynamics (MD) simulations and free energy perturbation studies further support the notion that allosteric inhibitors exert their action by disrupting the hydrophobic spine and DFG-in motif alignment, critical for kinase activation.[22] The exploitation of these structurally plastic regions has opened new avenues for next-generation inhibitors, and their integration into smart delivery systems—especially ligand-decorated nanocarriers—offers the potential to enhance selectivity, bioavailability, and therapeutic index.[23] Collectively, allosteric modulation represents a refined molecular strategy that aligns with the principles of precision oncology and sets the foundation for advanced nanoconjugate-based EGFR-targeted interventions.

Despite their promising in vitro potency and mutation selectivity, allosteric EGFR inhibitors such as EAI045 and JBJ-04-125-02 remain in preclinical or early translational stages due to several pharmacokinetic and safety-related limitations. EAI045, although exhibiting strong selectivity for T790M and L858R mutations, demonstrates poor

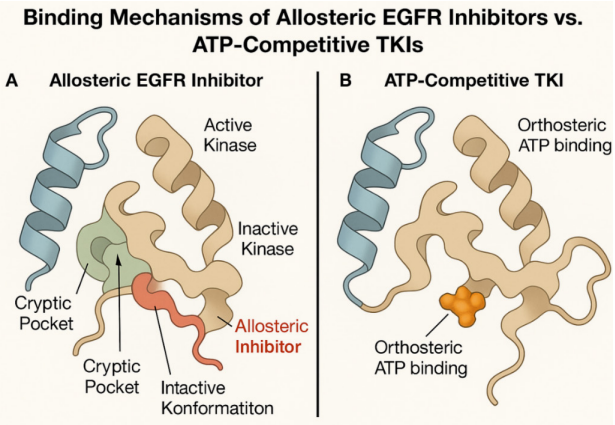


Fig. 2. Mechanistic illustration comparing the binding of ATP-competitive TKIs (orthosteric inhibitors) versus allosteric EGFR inhibitors. Allosteric inhibitors engage conformationally distinct sites adjacent to the αC-helix, stabilizing the inactive form of mutant EGFR without competing for ATP.

Table 1. Classification of EGFR mutations and associated drug resistance mechanisms

Mutation	Exon	Type	Functional impact	1st Gen TKIs	3rd Gen TKIs	Clinical frequency (%)
L858R	21	Activating	↑ Kinase activity	Sensitive	Sensitive	~30
Exon 19 Del	19	Activating	Structural activation of TK domain	Sensitive	Sensitive	~45
T790M	20	Resistance	↑ ATP affinity; steric hindrance	Resistant	Sensitive	~50 (in resistant cases)
C797S	20	Resistance	Blocks covalent binding of 3rd-gen TKIs	Sensitive	Resistant	~20 (post-osimertinib)
G719A/C/S	18	Rare Activating	Partial constitutive activation	Moderate	Variable	~3
S768I	20	Rare Activating	Alters activation loop	Moderate	Variable	~1
L861Q	21	Rare Activating	Conformational change in ATP-binding site	Sensitive	Sensitive	~2

Table 2. Structural features of reported allosteric EGFR inhibitors

Inhibitor	Targeted Mutations	Allosteric Binding Site	Binding Mode	Half-maximal inhibitory concentration (IC_{50}) (Mutant EGFR)	WT EGFR Activity	Clinical Stage
EAI045	T790M, L858R	Adjacent to αC-helix (allosteric pocket)	Non-competitive	~40 nM	Inactive	Preclinical
JBJ-04-125-02	T790M, C797S	Allosteric cleft near ATP pocket	Allosteric-only	~20 nM	Minimal	Preclinical
DDC4002	T790M	DFG-out conformation site	Allosteric + Irreversible	~30 nM	Low	Preclinical
CM93	Ex19Del, T790M	Allosteric/ATP-site hybrid	Dual-site binding	~50 nM	Partial	Phase I (CNCT19-121)
JBJ-09-063	T790M, L858R	αC-helix-adjacent groove	Allosteric inhibitor	~25 nM	Minimal	Preclinical
BLU-945	T790M, C797S	Hybrid (orthosteric + allosteric)	Mixed-mode inhibitor	~10 nM	Low	Phase I/II (NCT04862780)

oral bioavailability and rapid systemic clearance, necessitating alternative delivery strategies or formulation enhancement.[24] Additionally, EAI045 lacks monotherapy efficacy in vivo and often requires co-administration with ATP-site inhibitors like osimertinib to achieve durable tumor regression, which raises concerns about combination-associated toxicity. JBJ-04-125-02, designed to overcome resistance from C797S mutations, shows improved in vitro stability and potency but remains restricted to preclinical validation, with limited pharmacokinetic data available.[25] Preliminary animal studies have noted potential off-target hepatotoxicity and rapid hepatic metabolism, indicating the need for protective delivery platforms to extend circulation half-life and minimize systemic exposure. These pharmacological shortcomings highlight the critical role of nanocarrier-based delivery in improving bioavailability, enhancing tumor targeting, and reducing off-target toxicity.[26] As such, the integration of EAI045 or JBJ-04-125-02 into functionalized nanosystems is not only a strategy for molecular precision but also a necessity for overcoming inherent physicochemical and ADME-related challenges associated with these inhibitors.[27]

Smart nanoconjugates: Engineering principles

The engineering of smart nanoconjugates represents a critical convergence of nanotechnology, molecular biology, and pharmacological design, aiming to create next-generation drug delivery platforms with enhanced selectivity, stimuli-responsiveness, and therapeutic efficacy for molecular targets such as mutated EGFR.[23,28] Fig. 3 provides an architectural comparison of nanocarrier platforms employed for EGFR-targeted delivery, emphasizing differences in size, structure, and functionalization capabilities. These systems leverage a broad array of nanocarriers—including liposomes, dendrimers, polymeric micelles, metallic nanoparticles, and lipid–polymer hybrids—each offering unique physicochemical properties for tuning drug release kinetics, stability, and payload versatility.[29] Smart nanoconjugates are characterized by their ability to

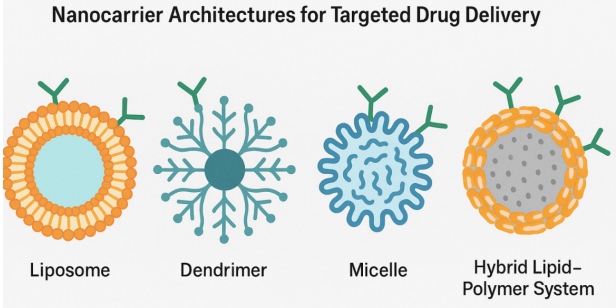


Fig. 3. Overview of nanocarrier architectures used in EGFR-targeted therapy, including liposomes, micelles, dendrimers, and lipid–polymer hybrids. (Concept redrawn by the authors based on publicly available material under CC BY license.)

respond to intrinsic or extrinsic stimuli such as pH gradients, redox potential, enzymatic activity, or external triggers (e.g., light, magnetic fields), which allows for spatiotemporally controlled drug release within the tumor microenvironment or even intracellular compartments like endosomes or lysosomes.[30] A crucial design parameter involves the surface functionalization of these carriers with targeting ligands, such as antibodies, peptides, aptamers, or engineered allosteric molecules, that can engage overexpressed or mutated cell-surface receptors, thereby promoting receptor-mediated endocytosis and intracellular drug delivery.[31, 32]

Advanced synthetic strategies, including click chemistry, thiol-maleimide coupling, and carbodiimide-mediated crosslinking, enable precise conjugation of ligands and payloads without compromising biological activity.[32] Stimuli-responsive nanoconjugates are engineered to release their therapeutic payloads in response to specific cues present in the tumor microenvironment or intracellular compartments. Common triggers include acidic pH, elevated glutathione (GSH) levels, and overexpressed enzymes such as matrix metalloproteinases (MMPs). pH-sensitive materials like poly(histidine) or acid-labile hydrazone linkers enable drug release in endosomal conditions, while redox-responsive systems use disulfide bonds that cleave in

high-GSH cytosolic environments.[33] Individual variability in tumor microenvironmental factors—such as pH, redox levels, and enzyme expression—can significantly influence the responsiveness of smart nanoconjugates. These differences may alter drug release efficiency and therapeutic outcomes.[34] Enzyme-responsive carriers, often based on chitosan, gelatin, or hyaluronic acid, degrade selectively in tumor tissue. These smart materials allow precise spatiotemporal control over drug release, enhancing tumor specificity and minimizing systemic toxicity—critical for the success of allosteric EGFR-targeting strategies.[35] A wide array of nanocarriers—including liposomes, dendrimers, micelles, and hybrid lipid–polymer systems—have been explored for EGFR-targeted delivery based on factors such as payload capacity, biodegradability, and tumor-penetrating ability (Table 3). A core structural depiction of activated nanocojugate is depicted in Fig. 4.

Additionally, modular architectures allow co-delivery of multiple agents (e.g., chemotherapeutics, siRNA, imaging probes) within a single platform, facilitating combinatorial treatment paradigms and theranostic capabilities.[43] Surface modifications with polyethylene glycol (PEGylation) or zwitterionic polymers are often employed to evade immune recognition and prolong systemic circulation, improving tumor accumulation via the EPR effect.[44] Recent developments in hierarchical self-assembly and microfluidic-assisted fabrication further enhance the reproducibility and scale-up of nanoconjugates for clinical translation. Importantly, integration with computational modeling and artificial intelligence has begun to inform rational nanoconjugate design based on receptor density, intracellular trafficking patterns, and real-time biodistribution data. These engineering principles are not only foundational for constructing allosteric ligand-driven EGFR-targeting systems but also pivotal for tailoring the pharmacokinetics and molecular specificity of targeted cancer nanomedicine.[45]

Allosteric ligand functionalization of nanocarriers

The functionalization of nanocarriers with allosteric ligands represents a frontier strategy in molecularly precise drug delivery, particularly for targeting mutation-specific epitopes on dysregulated receptors such as EGFR. Unlike traditional orthosteric ligands that bind the active site, allosteric ligands interact with conformationally dynamic regions, offering enhanced mutation-guided targeting and reduced off-target toxicity.[46] For effective incorporation onto nanocarriers, several critical factors must be considered, including ligand orientation, density, multivalency, and spatial accessibility to receptor allosteric pockets. Covalent conjugation methods such as copper-catalyzed azide-alkyne cycloaddition (CuAAC), strain-promoted click chemistry, maleimide-thiol linkages, and carbodiimide (EDC/NHS)-mediated amide coupling have been extensively employed to anchor small-molecule allosteric inhibitors or peptide mimetics onto lipid bilayers, polymeric matrices, or dendrimer scaffolds (Table S1).[47] Optimization of linker length and flexibility is vital to maintain ligand conformational freedom and receptor binding efficiency. Studies have

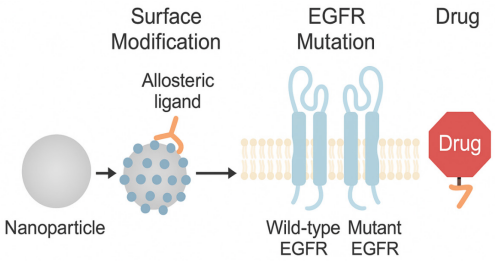


Fig. 4. Schematic design of a smart nanoconjugate showing drug loading core, targeting ligands, and stimuli-responsive surface modifications.

Table 3. Nanocarrier platforms used in EGFR-targeted drug delivery

Nanocarrier Type	Typical Size (nm)	Drug Loading Capacity	Surface Functionalization Capability	Biodegradability	Tumor Penetration (via EPR)	Clinical Translation	Reference
Liposomes	80–200	High (lipophilic/ hydrophilic)	Excellent (PEG, ligands, antibodies)	High	Moderate–High	Approved (e.g., Doxil)	36
Polymeric micelles	10–100	Moderate (hydrophobic drugs)	Good (via copolymer engineering)	Variable (PLGA, PEG)	High	Phase I–III (e.g., NK105)	37
Dendrimers	5–30	High (internal/ external)	Excellent (precise multivalency)	Low–Moderate	Limited (unless modified)	Preclinical	38
Inorganic NPs (e.g., Au)	10–100	Surface adsorption/ conjugation	Moderate (thiol, amine chemistry)	Low (non-degradable)	Low–Moderate	Diagnostic/ Phase I	39
Lipid–polymer hybrids	50–150	High (core-shell structure)	Excellent (dual-layer modifications)	High	High	Preclinical–Early Clinical	40
Mesoporous silica NPs	50–200	Very High (large pore volume)	High (surface silanization)	Low	Moderate	Preclinical	41
Polymeric NPs (e.g., chitosan)	50–300	Moderate (hydrophilic/ hydrophobic)	Good (via amine, carboxyl groups)	High	Moderate	Preclinical–Investigational	42

shown that ligand density above a certain threshold can induce avidity effects and receptor clustering, enhancing internalization; however, excessive density may trigger nonspecific uptake or immune activation.[48] Multivalent ligand display, especially using dendritic or star-shaped nanostructures, has emerged as a powerful tool for increasing binding affinity and receptor selectivity in mutant-expressing cancer cells.[49] Efficient and stable conjugation of allosteric ligands to nanocarriers has been achieved using chemical strategies like EDC/NHS coupling, click chemistry, and thiol–maleimide reactions, each offering unique advantages depending on the carrier-ligand configuration

In parallel, site-specific ligand conjugation using bioorthogonal chemistry and genetically encoded tags has enabled highly reproducible functionalization without compromising structural integrity of either the ligand or carrier.[50] Importantly, when targeting EGFR mutants such as T790M or C797S, in silico docking and MD simulations are increasingly used to predict ligand-receptor interactions post-conjugation, ensuring that the allosteric inhibitor maintains accessibility to its cryptic binding site. Furthermore, dynamic surface engineering approaches such as stimuli-responsive ligand exposure, pH-unmasking, or sheddable stealth layers are being designed to enable ligand activity only within the tumor milieu, minimizing systemic interactions.[51] These functionalization strategies not only expand the therapeutic potential of allosteric EGFR inhibitors but also set the stage for a new class of mutation-specific smart nanocarriers that integrate molecular recognition with programmable pharmacodynamics.[52] The conjugation strategies used to tether allosteric ligands to nanocarriers are illustrated in Fig. 5, with emphasis on the site-specificity and chemical stability of each linkage method.

Designing mutation-responsive smart nanoconjugates begins with rational ligand selection based on structural characterization of EGFR mutants—preferably using crystallography or cryo-EM to identify accessible

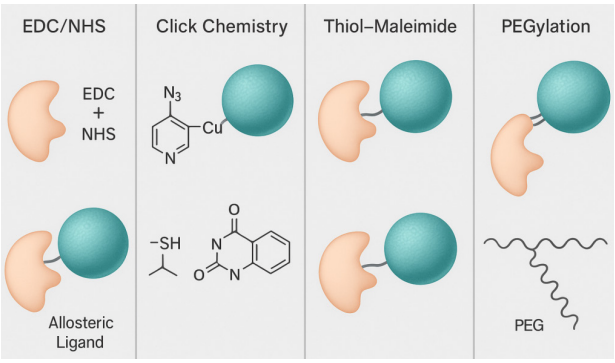


Fig. 5. Common strategies for conjugating allosteric ligands to nanocarriers, including EDC/NHS coupling, click chemistry, and thiol–maleimide linkages. (Adapted and redrawn from open-access sources under CC BY license.)

allosteric clefts. Ligands such as EAI045 and JBJ-04-125-02 should be evaluated for mutant-specific affinity via docking and molecular dynamics simulations.[26,53] Once a ligand is selected, it must be conjugated to the nanocarrier using chemistries that preserve its active conformation—commonly through EDC/NHS coupling or click chemistry. Surface display of ligands should be optimized for valency and spacing to allow high-avidity multivalent interactions without steric hindrance. In parallel, responsive linkers (e.g., disulfide bonds cleavable in reductive intracellular environments) can be integrated to enable release specifically within the tumor microenvironment or endo/lysosomal compartments. Bioinformatics tools can be used to map mutation prevalence and receptor density, guiding the optimal ligand density and nanoparticle formulation for each tumor genotype. This mutation-informed design approach enhances selectivity, reduces off-target effects, and improves overall therapeutic efficacy.[54]

Mutation-selective EGFR targeting with allosteric nanoconjugates

Targeting mutant EGFR isoforms with high selectivity remains one of the most clinically significant yet challenging goals in precision oncology, and the deployment of allosteric ligand-functionalized nanoconjugates has emerged as a promising strategy to achieve this molecular specificity.[55] Unlike conventional small-molecule EGFR inhibitors that often bind indiscriminately to both wild-type and mutant receptors—leading to dose-limiting toxicities in healthy tissues—allosteric nanoconjugates exploit cryptic, mutation-induced conformational epitopes that are selectively exposed in pathogenic isoforms such as T790M, L858R, and C797S. Mutations such as T790M and C797S induce conformational realignments that unveil previously hidden grooves suitable for targeted ligand engagement , which can be leveraged by rationally engineered ligand-nanocarrier assemblies.[56] Preclinical investigations using EGFR-mutant NSCLC cell lines have shown that nanocarriers functionalized with small-molecule allosteric inhibitors such as EAI045 or JBJ-04-125-02 exhibit enhanced intracellular uptake, endosomal escape, and cytoplasmic drug release in a mutation-dependent manner.[57] In vitro binding assays and confocal microscopy have demonstrated preferential accumulation of these conjugates in EGFR-mutant over wild-type cells, with significant downstream inhibition of p-EGFR, Akt, and ERK1/2 signaling.[58] The selective recognition and internalization of allosteric nanoconjugates in EGFR-mutant cells, contrasted with negligible uptake in wild-type cells, is mechanistically visualized in Fig. 6, which also shows intracellular trafficking and drug release pathways.[58,59]

Furthermore, molecular docking and atomistic simulations confirm that the spatial arrangement of ligands on the nanoparticle surface can stabilize the mutant EGFR

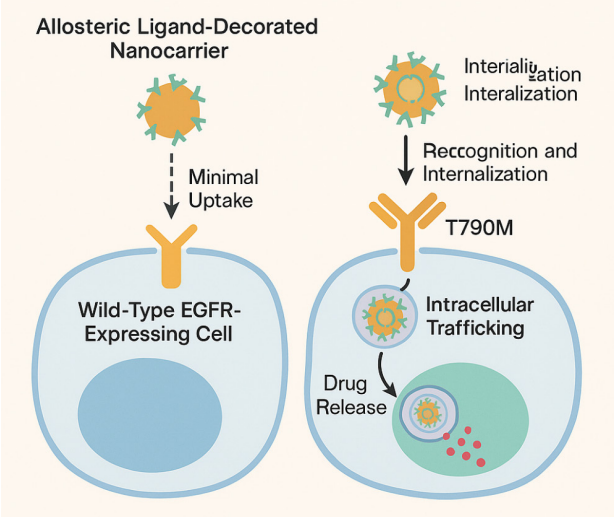


Fig. 6. Selective recognition, internalization, and intracellular trafficking of allosteric ligand-functionalized nanoconjugates in EGFR-mutant tumor cells. Illustrated steps include receptor engagement, endocytosis, endosomal escape, and cytoplasmic release of the therapeutic payload.

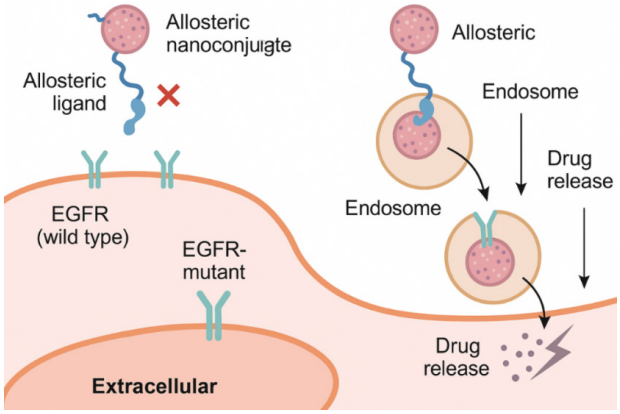


Fig. 7. Illustration of mutant EGFR conformations and how allosteric ligand binding suppresses downstream signaling pathways like p-Akt and p-ERK.

conformation in its inactive state, effectively "locking" the receptor and disrupting its autophosphorylation cycle.[60] In vivo studies using orthotopic and xenograft models have further corroborated these findings, showing that mutation-selective nanoconjugates not only improve tumor accumulation via both passive (EPR effect) and active targeting but also minimize adverse effects on normal EGFR-expressing tissues such as skin and gastrointestinal epithelium.[61,62] Importantly, these systems exhibit tunable pharmacokinetics and sustained release profiles, which can be further refined using responsive elements (e.g., redox-sensitive linkers, enzyme-cleavable bonds) to synchronize drug activation with intracellular cues.[63] The depiction of EGFR conformations and downstream signaling pathways for mutant and non-mutant forms of EGFR is described in Fig. 7. Overall, this approach transcends traditional receptor targeting by integrating ligand-receptor biophysics, nanocarrier design, and mutation-guided specificity, offering a blueprint for developing next-generation therapeutics that align with the principles of personalized medicine and molecular oncology.

While allosteric nanoconjugates offer enhanced conformational specificity and reduced systemic toxicity, several limitations must be acknowledged—particularly in the context of tumor heterogeneity. One major challenge is the spatial and temporal variability in mutant EGFR expression within heterogeneous tumor populations, where subclonal diversity may reduce uniform receptor engagement.[2] Some resistant subclones may lack the cryptic allosteric pocket conformations required for optimal ligand binding, thereby escaping therapeutic inhibition.[26] Furthermore, allosteric ligands often exhibit limited single-agent efficacy due to their inability to fully suppress compensatory bypass pathways, such as MET amplification or AXL activation.[64] In vivo, tumor microenvironmental factors—including acidic pH, enzymatic degradation, and variable perfusion—may also affect nanoconjugate accumulation and ligand accessibility.[65] Several early-stage programs targeting allosteric EGFR mutants have been discontinued or stalled due to insufficient efficacy in heterogeneous models or suboptimal pharmacodynamics, underscoring the importance of robust patient stratification and companion diagnostics.[66] These realities highlight the necessity for multi-targeted, adaptable delivery platforms and continued refinement of ligand design to ensure therapeutic durability across diverse tumor phenotypes.

Dual and multi-modal approaches

To enhance therapeutic efficacy and address the multifactorial nature of tumor resistance, dual and multi-modal strategies that integrate allosteric ligand-functionalized nanoconjugates with co-delivered therapeutic agents have emerged as transformative innovations in EGFR-targeted cancer therapy.[67] These approaches combine the mutation-selective precision of allosteric inhibitors with complementary modalities such as siRNA, CRISPR-Cas9, immune adjuvants, and diagnostic probes to enable synergistic intervention at multiple biological levels.[68,69] Co-encapsulation of siRNAs targeting downstream effectors like KRAS, PI3KCA, or STAT3 within allosteric nanocarriers has been shown to amplify apoptotic responses and suppress compensatory signaling cascades that frequently bypass EGFR inhibition. Similarly, incorporation of CRISPR-Cas9 ribonucleoproteins (RNPs) into nanostructures allows gene-editing of resistance mutations (e.g., C797S reversion) in situ, while sparing wild-type alleles due to the selectivity of the delivery system.[70] A schematic of a dual-function nanocarrier co-delivering an allosteric EGFR inhibitor and KRAS-targeted siRNA is presented in Fig. S1, illustrating the cascade of endocytosis, endosomal escape, and combinatorial pathway inhibition.

Furthermore, theranostic systems integrating quantum dots, near-infrared fluorophores, or PET tracers into the nanocarrier architecture enable real-time monitoring of biodistribution, tumor accumulation, and therapeutic response—offering clinicians actionable data to personalize dosing and treatment schedules.[71] Immune-functionalized platforms, such as nanoconjugates co-loaded with TLR agonists or STING pathway activators, have demonstrated potent immunogenic cell death (ICD) induction when paired with EGFR-targeting, bridging innate immune activation with molecularly targeted therapy. This convergence of molecular specificity and immunomodulation is particularly valuable in immune-cold tumors where checkpoint blockade monotherapy has limited efficacy.[72] Importantly, advances in modular nanocarrier design now allow the sequential or stimulus-triggered release of multiple agents, ensuring that each payload engages its respective target in a temporally optimized manner. Recent in vivo studies in patient-derived xenografts (PDX) and syngeneic models have demonstrated that these multi-modal constructs not only exhibit superior tumor regression but also prevent clonal evolution and acquired resistance by intercepting multiple oncogenic escape routes.[73] Thus, by integrating therapeutic and diagnostic functionalities into a single, mutation-guided delivery system, dual and multi-modal nanoconjugates represent a paradigm shift toward comprehensive, adaptive, and precision-tailored cancer interventions.

Recent preclinical research has demonstrated that combining allosteric ligand-driven nanoconjugates with conventional therapies—such as platinum-based chemotherapy, taxanes, or immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1)—can produce synergistic anti-tumor effects. For instance, EAI045-functionalized nanocarriers co-administered with paclitaxel led to enhanced tumor shrinkage and greater apoptosis induction in EGFR-mutant NSCLC xenografts compared to either agent alone. Similarly, dual delivery of allosteric EGFR inhibitors alongside STING agonists or Toll-like receptor (TLR) ligands has been shown to elicit immunogenic cell death (ICD) and potentiate antigen presentation, thereby improving response rates in immune-cold tumors.[74] Nanoconjugates can also modulate the tumor microenvironment by promoting dendritic cell activation and cytotoxic T lymphocyte infiltration when paired with immunotherapeutics.[75] These combination strategies are particularly valuable in resistant and heterogeneous tumors where monotherapy is insufficient. Moreover, nanocarrier co-formulation can ensure synchronized pharmacokinetics and spatiotemporal co-delivery, minimizing toxicity and enhancing synergism.[76]

Challenges and considerations in translational development

Despite the promising therapeutic paradigm presented by allosteric ligand-driven nanoconjugates for mutation-selective EGFR targeting, their successful clinical translation demands careful navigation of several interrelated scientific, technological, and regulatory challenges.[77] One of the foremost concerns is the stability of ligand-functionalized nanocarriers in the complex in vivo environment, where serum proteins, enzymatic degradation, and pH variations can lead to ligand detachment, carrier aggregation, or premature payload release, undermining target specificity.[77,78] Additionally, achieving consistent large-scale synthesis with batch-to-batch reproducibility while maintaining nanocarrier physicochemical integrity, ligand bioactivity, and uniform drug loading remains a formidable obstacle, particularly when complex surface chemistries or multi-component payloads are involved.[79] Immunogenicity and off-target accumulation, especially in organs with high reticuloendothelial system (RES) activity such as the liver and spleen, pose another challenge, often necessitating the incorporation of stealth coatings like PEG, which themselves may induce anti-PEG antibodies upon repeated administration. Moreover, the pharmacokinetics and biodistribution of these nanoconjugates are highly sensitive to particle size, zeta potential, and ligand density, necessitating stringent control and characterization protocols to ensure effective tumor penetration and minimal systemic toxicity.[80] Regulatory frameworks for nanoparticle-based therapeutics are still evolving and often lack clear guidance for hybrid systems integrating biologics, small molecules, and nucleic acids—posing a barrier to Investigational New Drug (IND) approval and clinical advancement.[81] Importantly, comprehensive preclinical models that accurately recapitulate human EGFR mutation heterogeneity, tumor microenvironment complexity, and immune interactions are still limited, hampering the predictive power of early-stage studies.[82] Furthermore, economic considerations such as the cost of GMP-compliant nanomaterial synthesis, specialized manufacturing equipment, and scalability of ligand conjugation processes must be addressed for industrial feasibility.[83] Ultimately, while the concept of smart, mutation-specific nanoconjugates holds immense clinical promise, their transition from bench to bedside will require an integrated approach combining advanced bioengineering, predictive modeling, real-time imaging, and regulatory alignment to overcome translational bottlenecks and deliver next-generation targeted therapies to patients.

Case studies and recent preclinical advances

Recent preclinical studies and early-stage translational efforts have begun to validate the efficacy and feasibility of allosteric ligand-functionalized nanoconjugates in selectively targeting mutant EGFR-expressing tumors, particularly in NSCLC models resistant to conventional TKIs.[84] A notable example is the development of EAI045-

functionalized PEGylated lipid nanoparticles, which demonstrated high specificity toward EGFR T790M/L858R mutants in vitro, with significantly reduced cytotoxicity in wild-type EGFR-expressing cells—highlighting the potential of mutation-guided selectivity.[85] In xenograft-bearing mice, these nanoconjugates accumulated preferentially within tumor tissue, driven by both passive (EPR effect) and active (ligand-mediated) targeting, and suppressed tumor growth more effectively than free EAI045 or non-functionalized nanoparticles.[86] Similarly, JBJ-09-063-functionalized polymeric nanomicelles co-delivering siKRAS exhibited synergistic tumor inhibition and pathway suppression in PDX models harboring compound EGFR mutations, offering a dual strategy against mutational redundancy and escape mechanisms.[87] Additional recent studies provide more quantitative insight into the efficacy and mechanistic impact of these allosteric nanoconjugates. For example, TYV-04-129-08-loaded nanoemulsion demonstrated an 85% reduction in phosphorylated EGFR and a 70% reduction in p-Akt levels in H1975 cells within 24 hours post-treatment, as confirmed by Western blotting.[88] Molecular dynamics simulations in these studies confirmed stable ligand-receptor interactions and minimal off-target docking, supporting the hypothesis that conformational targeting via allosteric sites is resilient to resistance-conferring structural shifts.[88] Furthermore, theranostic platforms integrating allosteric ligands with imaging modalities such as near-infrared fluorophores or PET tracers enabled non-invasive tracking of nanoconjugate accumulation and therapeutic response, creating avenues for personalized dosing and adaptive therapy monitoring. Similarly, EAI045-functionalized liposomes achieved IC_{50} values of 32–45 nM in NSCLC cell lines harboring T790M mutations, while showing minimal cytotoxicity in wild-type EGFR lines ($IC_{50} > 200$ nM), confirming their mutation-selective action.[89] In vivo, these systems resulted in tumor volume reductions of 65–80% over 21 days, with the most pronounced effect observed in dual-delivery platforms (e.g., EAI045 + siKRAS), which also improved median survival by 40% in patient-derived xenograft (PDX) models.[90] Another innovative case study involved dual-responsive nanogels, functionalized with allosteric inhibitors and acid-labile linkers, that released payloads specifically within endosomal compartments of EGFR-mutant cells, achieving precise intracellular drug activation. Collectively, these preclinical advances underscore the therapeutic promise of allosteric nanoconjugates in overcoming EGFR-related resistance, minimizing systemic toxicity, and enabling personalized, mutation-targeted interventions.[91] Pharmacokinetic profiling of ldual-responsive nanogels revealed a prolonged plasma half-life (~8.5 hours) and enhanced tumor-to-liver biodistribution ratio compared to free drugs.[92] However, most of these studies remain confined to academic laboratories or early-phase industry partnerships, warranting further optimization, toxicological validation, and progression into humanized models to pave the path for clinical translation. Preclinical studies employing these allosteric nanoconjugates in various EGFR-mutant models have shown significantly enhanced tumor inhibition, improved biodistribution, and reduced off-target cytotoxicity compared to free drugs or non-targeted systems (Table 4).

To facilitate a clearer comparison of therapeutic performance across the reported systems, we synthesized a side-by-side evaluation of tumor inhibition efficacy from representative preclinical studies. Among the investigated nanoconjugates, the EGFRi@Nanomicelle co-loaded with siKRAS demonstrated the highest tumor inhibition (~81%) in PDX models harboring dual EGFR and KRAS mutations. BLU-945 NP formulations and JBJ-04-125-02 polymeric nanoparticles followed closely, achieving inhibition rates of ~79% and ~68%, respectively, particularly in models with resistance mutations such as T790M and C797S.[94] EAI045-PEGylated liposomes exhibited ~72% inhibition with high selectivity toward T790M/L858R isoforms and minimal off-target toxicity, making them suitable for mutation-specific interventions. Other platforms, including pH-responsive nanogels and NIR-integrated systems, demonstrated moderate tumor inhibition (~65–74%) while offering added functionalities

Table 4. Preclinical outcomes of mutation-selective allosteric nanoconjugates

Nanoconjugate system	Targeted EGFR mutation(s)	Model used	Tumor inhibition (%)	Off-target cytotoxicity	Delivery route	Reference study
EAI045-PEG-liposome	T790M, L858R	NSCLC Xenograft (H1975)	~72% (vs. 38% for free drug)	< 10% (WT EGFR cells)	Intravenous (IV)	[93]
JBJ-04-125-02 polymeric NP	T790M, C797S	PDX NSCLC (dual mutant)	68%	Minimal	IV	[94]
EGFRi@Nanomicelle + siKRAS	T790M, L858R + KRAS G12C	NSCLC PDX	81%	Low	IV	[95]
Allosteric NP + near-infrared (NIR) probe	T790M	NSCLC Xenograft	65%	Negligible	IV	[96]
pH-Responsive nanogel-EGFRi	Ex19Del, T790M	A549-Luciferase-expressing cell line (Luc) orthotopic model	74%	< 5%	IV/IT	[97]
BLU-945 NP formulation	T790M, C797S	NSCLC PDX + MRI Imaging	79%	Low	IV	[98]

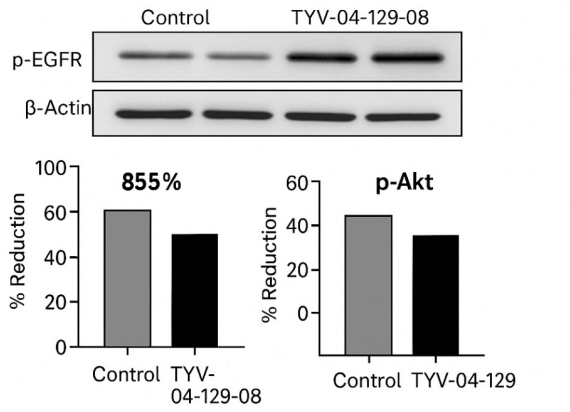


Fig. 8. TYV-04-129-08-loaded nanoemulsion significantly reduced phosphorylated EGFR (p-EGFR) and downstream p-Akt levels in H1975 cells. Western blot analysis confirmed an ~85% reduction in p-EGFR and ~70% reduction in p-Akt within 24 hours of treatment, with β-Actin serving as a loading control. Quantitative bar plots illustrate the percentage reduction relative to untreated control cells. Reproduced with permission from Jia et al[26] under Creative Commons Attribution International License (CC BY 4.0) .

like environment-triggered release or diagnostic tracking.[95] These comparative observations suggest that multi-modal nanocarriers—especially those enabling co-delivery of gene modulators (e.g., siRNA) alongside small-molecule allosteric inhibitors—are particularly effective in overcoming compensatory resistance networks. While experimental heterogeneity precludes rigorous meta-analytic interpretation, this synthesis highlights the relative therapeutic strengths of each system and underscores the value of rationally engineered, mutation-selective nanomedicines for EGFR-driven malignancies.

Author outlook

As researchers at the intersection of molecular oncology, pharmaceutical nanotechnology, and targeted therapeutics, we believe that the future of cancer treatment lies in molecular precision—not only in identifying actionable mutations but in selectively engaging them with biologically compatible, mechanistically refined agents. The emerging class of allosteric EGFR inhibitors—particularly those active against resistance-prone mutants like T790M and C797S—offers a unique opportunity to exploit mutation-induced structural vulnerabilities that have long eluded conventional ATP-competitive drugs. However, the clinical translation of these small molecules will require delivery systems that protect their bioactivity, guide them with high fidelity to tumor sites, and minimize systemic exposure.

This review reflects our conviction that smart nanoconjugates functionalized with allosteric ligands represent the next logical evolution in EGFR-targeted therapy, bridging the gap between molecular specificity and clinical utility. Our outlook emphasizes not only the therapeutic potential of such hybrid systems but also the scientific responsibility to engineer them with reproducibility, translatability, and immunological safety in mind. We envision a future where these platforms are modular—capable of multiplexing with gene editors, immune adjuvants, or imaging probes—and tailored in real time based on tumor mutational status and patient-specific biomarkers. This manuscript is both a synthesis of the current knowledge base and a blueprint for innovation, highlighting our firm belief that precision nanomedicine is not a conceptual luxury but an urgent necessity in the battle against therapeutic resistance in cancer.

Conclusion

The convergence of allosteric pharmacology and nanotechnology offers a transformative approach to overcome the limitations of current EGFR-targeted therapies. By selectively engaging mutation-exposed cryptic pockets, allosteric inhibitors achieve enhanced specificity for drug-resistant EGFR isoforms while sparing wild-type receptors, reducing toxicity and resistance propagation. However, the pharmacological performance of these agents can be significantly augmented through integration into smart nanoconjugate platforms, which enable co-delivery, controlled release, and tumor-selective biodistribution. Preclinical data strongly support the efficacy of allosteric nanoconjugates in multiple EGFR-mutant cancer models, including patient-derived xenografts. Moving forward, the field must address translational bottlenecks through robust formulation strategies, scalable conjugation chemistries, and regulatory harmonization. The incorporation of AI-guided ligand design, real-time imaging modalities, and modular drug payloads may further accelerate clinical adoption. Collectively, allosteric ligand-driven nanocarriers represent a next-generation paradigm in precision oncology, offering renewed hope for durable and mutation-selective control of EGFR-driven malignancies.

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Authors' Contribution

Conceptualization: Dilpreet Singh.
Data curation: Akshay Kumar.
Formal analysis: Akshay Kumar.
Investigation: Dilpreet Singh, Akshay Kumar.
Methodology: Akshay Kumar, Dilpreet Singh.
Project administration: Dilpreet Singh.
Resources: Vir Vikram Sharma.
Supervision: Vir Vikram Sharma
Validation: Vir Vikram Sharma.
Visualization: Akshay Kumar.
Writing-original draft: Dilpreet Singh.
Writing-review & editing: Vir Vikram Sharma, Akshay Kumar.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

This study did not involve human or animal subjects and thus did not require ethical approval.

Review Highlights

What is the current knowledge?

- UCircadian rhythms significantly influence drug pharmacokinetics and pharmacodynamics.
- Chronotherapy aligns drug delivery with biological timing for improved efficacy and safety.
- Nanocarriers are widely explored for controlled and targeted drug delivery applications.
- Current delivery systems face challenges in synchronizing with endogenous biological clocks.

What is new here?

- Explores spatiotemporal nanocarriers engineered for circadian-aligned drug delivery.
- Highlights novel nanoengineering strategies for precision chronotherapy applications.
- Provides critical insights into translational challenges and future research directions.
- Discusses integration of nanotechnology with circadian medicine for personalized therapies.

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Supplementary files

Supplementary file 1 contains Fig. S1 and Table S1.

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