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RESEARCH PAPER

Lipid Nanoparticles Carrying Gemcitabine and Hyaluronidase for Simultaneous Targeting Of Stroma and Pancreatic Cancer Cells: To Overcome Drug Resistance and Improve Permeability: A Review

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ABSTRACT

Pancreatic cancer's aggressive biology—marked by drug-resistant phenotypes and a desmoplastic stroma—has long presented formidable barriers to effective treatment. Gemcitabine, a cornerstone chemotherapy for this malignancy, faces clinical limitations due to poor tissue penetration and diminished therapeutic efficacy in the face of these physiological hurdles. Hyaluronidase, an enzyme that disrupts the stromal matrix to enhance drug delivery, offers a partial solution but is constrained by suboptimal targeting and risks of nonspecific tissue effects. Emerging as a transformative approach, multifunctional lipid nanoparticles (LNPs) co-encapsulating gemcitabine and hyaluronidase now promise to address these dual challenges synergistically. Engineered with pH-responsive properties, these "smart" LNPs exploit the acidic tumor microenvironment to achieve spatiotemporally controlled release, enhancing stromal degradation while maximizing intracellular gemcitabine delivery. Preclinical studies leveraging Patient-Derived Xenograft (PDX) models have shown remarkable tumor growth suppression, underscoring the potential of this combinatorial platform. Nevertheless, key challenges persist, including optimizing nanoparticle stability in protease-rich environments, refining hyaluronidase dosing to balance stromal modulation with off-target toxicity, and ensuring scalable manufacturing. This review critically examines recent breakthroughs in stimuli-responsive LNP design, evaluates translational gaps through the lens of clinical applicability, and proposes forward-looking strategies to advance this paradigm toward personalized, stroma-targeted therapy for pancreatic cancer.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) persists as a therapeutic enigma, with a five-year survival rate below 10%, driven by its late-stage detection, intrinsic chemoresistance, and uniquely hostile tumor microenvironment (TME) [1]. Unlike most solid tumors, PDAC is defined by a desmoplastic stroma—a fibroinflammatory matrix enriched in hyaluronic acid (HA), collagen, and cancer-associated fibroblasts (CAFs) [2]. This dynamic barrier not only restricts vascular perfusion and drug diffusion but also fosters immunosuppression, hypovascularity, and adaptive survival signaling in tumor cells. Consequently, conventional chemotherapies, including the nucleoside analog gemcitabine, exhibit suboptimal bioavailability and transient efficacy [3]. Gemcitabine, the first-line therapy for PDAC, suffers from rapid systemic deamination, poor tumor accumulation (<0.1% injected dose), and resistance mechanisms such as reduced expression of nucleoside transporters (hENT1) and deoxycytidine kinase (dCK) [4]. While stromal modulation strategies—notably PEGylated hyaluronidase (PEGPH20)—have shown promise in degrading HA to normalize interstitial fluid pressure and improve perfusion, their clinical utility is curtailed by dose-limiting systemic toxicity (e.g., musculoskeletal events from off-target extracellular matrix (ECM) degradation) and incomplete penetration into stromal niches [5].

This review delineates a paradigm-shifting approach: stimuli-responsive lipid nanoparticles (LNPs) engineered for tumor-selective co-delivery of gemcitabine and hyaluronidase. By leveraging the acidic pH gradient of the PDAC TME (pH 6.5–7.0 vs. physiological 7.4), these LNPs employ ionizable lipids or pH-labile linkers to achieve spatiotemporally controlled release. This dual-loading strategy synchronizes stromal HA depletion with intracellular gemcitabine delivery, circumventing systemic hyaluronidase toxicity while potentiating chemotherapeutic uptake. Mechanistically, HA degradation disrupts CD44-mediated oncogenic signaling and enhances vascular patency, creating a feedback loop that amplifies nanoparticle penetration and tumor cell apoptosis.

Through a critical synthesis of preclinical evidence—including orthotopic PDX models, intravital imaging, and single-cell RNA sequencing—we evaluate how these LNPs overcome multidrug resistance (MDR) transporters, CAF crosstalk, and hypoxic niches. Key challenges, such as enzymatic degradation of LNPs in protease-rich stroma, burst release in systemic circulation, and scalable synthesis of hyaluronidase-loaded vesicles, are analyzed through a translational lens. By integrating pharmacokinetic modeling, biocompatibility assessments, and combinatorial regimens (e.g., immune checkpoint inhibitors), this review outlines a roadmap for clinical translation, positioning pH-responsive LNPs as a multimodal platform to dismantle PDAC's stromal fortress.

REVIEW OF LIMITATIONS OF GEMCITABINE AND HYALURONIDASE ALONE

Gemcitabine

Gemcitabine (2',2'-difluorodeoxycytidine) remains the cornerstone of chemotherapy for pancreatic ductal adenocarcinoma (PDAC), yet its clinical efficacy is profoundly constrained by multifaceted barriers [6]. These limitations arise from both the unique pathophysiology of PDAC and the drug's inherent pharmacokinetic and pharmacodynamic properties [7]. PDAC is characterized by a dense, fibrotic stroma that constitutes up to 80% of the tumor volume. This stromal compartment is composed of a hypersecretory extracellular matrix (ECM) enriched in hyaluronic acid (HA), collagen types I and III, fibronectin, and laminin, alongside a heterogeneous population of cancer-associated fibroblasts (CAFs), immune cells, and endothelial cells [8]. The ECM's structural rigidity is further amplified by covalent cross-linking mediated by enzymes such as lysyl oxidase (LOX), which enhances collagen fibril stability. This fibrotic network functions as a "stromal shield," physically compressing blood vessels and reducing vascular density by over 70% compared to normal pancreatic tissue [9]. The consequent elevation in interstitial fluid pressure (IFP)—reaching 30–100 mmHg in PDAC versus <10 mmHg in most solid tumors—creates a hemodynamic barrier to drug delivery. High IFP collapses tumor vasculature, impairing gemcitabine perfusion and trapping the drug in the stroma [10]. Studies using radiolabeled gemcitabine reveal that less than 0.1% of the administered dose penetrates tumor cells, with the majority sequestered in peritumoral stroma [11]. Furthermore, the hypoxic microenvironment induced by poor perfusion activates hypoxia-inducible factors (HIF-1α/2α), upregulating

survival pathways like glycolysis and autophagy in cancer cells, further diminishing gemcitabine's cytotoxic effects [12]. CAFs exacerbate these challenges by secreting ECM components and growth factors (e.g., TGF-β, PDGF) that sustain stromal density. Notably, CAF-derived exosomes transfer microRNAs (e.g., miR-21, miR-155) to tumor cells, enhancing their chemoresistance [13]. The stroma also restricts lymphatic drainage, prolonging the retention of cytotoxic metabolites in healthy tissues and increasing off-target toxicity [14]. Gemcitabine's pharmacokinetic profile further limits its efficacy. The drug is rapidly metabolized by cytidine deaminase (CDA), an enzyme ubiquitously expressed in the liver, plasma, and peripheral tissues [15]. Within 15 minutes of administration, over 90% of gemcitabine is deaminated to its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), resulting in a plasma half-life of <15 minutes. This necessitates frequent, high-dose infusions (e.g., 1000 mg/m² weekly), which escalate toxicity risks [16]. Genetic polymorphisms in CDA significantly influence interpatient variability. For instance, the CDA2 allele, present in 10–20% of populations, reduces enzymatic activity, leading to prolonged drug exposure and severe hematologic toxicity [17]. Conversely, CDA overexpression in tumors accelerates gemcitabine inactivation, contributing to resistance. Hepatic metabolism further complicates this picture, as dFdU accumulation correlates with hepatotoxicity, manifesting as elevated transaminases and steatosis in 30–40% of patients [18]. Myelosuppression, particularly neutropenia and thrombocytopenia, necessitating dose reductions or delays [19]. This stems from gemcitabine's impact on bone marrow progenitors, where it inhibits ribonucleotide reductase (RNR), depleting deoxyribonucleotide pools essential for DNA repair in hematopoietic cells [20]. Gemcitabine relies on human equilibrative nucleoside transporter 1 (hENT1/SLC29A1) for cellular entry [21]. However, PDAC tumors exhibit epigenetic silencing or downregulation of hENT1 due to promoter hypermethylation or miR-21-mediated post-transcriptional repression [22]. Intracellular activation of gemcitabine requires phosphorylation by deoxycytidine kinase (dCK). However, dCK expression is suppressed in resistant PDAC via promoter methylation or transcriptional repression by oncogenic KRAS [23]. Over 90% of PDAC tumors harbor KRAS mutations (e.g., G12D, G12V), which constitutively activate downstream pathways like RAF/MEK/ERK and PI3K/AKT [24]. These pathways enhance survival by upregulating anti-apoptotic proteins (Bcl-2, Mcl-1) and DNA repair enzymes (PARP, BRCA1). Simultaneously, TP53 mutations (75% of PDAC) disrupt pro-apoptotic signaling, enabling cells to bypass cell cycle checkpoints. Mutant p53 also transcriptionally activates hyaluronan synthase 2 (HAS2), perpetuating stromal HA production and IFP [25].

Gemcitabine-induced DNA damage activates stress kinases (ATM/ATR), which stabilize HIF-1α and NF-κB. These transcription factors upregulate survivin, XIAP, and c-FLIP, conferring resistance to apoptosis [26]. Additionally, gemcitabine triggers autophagy via AMPK/ULK1 signaling, enabling tumor cells to recycle damaged organelles and sustain metabolic demands during treatment [27]. While enzymatic degradation of HA with hyaluronidase (e.g., PEGPH20) reduces stromal HA and transiently lowers IFP, clinical trials have shown limited success [28]. PEGPH20 monotherapy fails to address collagen cross-linking, CAF activation, and compensatory ECM remodeling via fibronectin and Tenascin-C upregulation [29, 30]. Moreover, HA degradation releases pro-angiogenic fragments (e.g., hyaluronan oligosaccharides), which paradoxically accelerate metastasis in preclinical models [31]. The limitations of gemcitabine and hyaluronidase in PDAC underscore the need for multimodal strategies targeting stromal remodeling, metabolic adaptation, and oncogenic signalling [32].

Hyaluronidase

Recombinant PEGylated hyaluronidase (PEGPH20) is engineered to degrade hyaluronic acid (HA), a major component of the tumor stroma in pancreatic ductal adenocarcinoma (PDAC) [33]. By cleaving HA into smaller fragments, PEGPH20 reduces stromal rigidity and interstitial fluid pressure (IFP) by 40–60% in preclinical models, transiently improving vascular perfusion and gemcitabine delivery [34]. However, as a monotherapy, hyaluronidase faces significant limitations, ranging from off-target toxicity to stromal adaptation and failure to address intrinsic tumor resistance [35]. HA is a critical structural and signaling molecule in healthy tissues, including synovial fluid, dermis, and the extracellular matrix (ECM) of organs like the liver and kidneys [36].

Systemic administration of PEGPH20 disrupts HA homeostasis in these tissues, leading to dose-dependent adverse effects [37]. These symptoms arise from HA degradation in synovial fluid, which reduces joint lubrication and increases friction between cartilage surfaces. Similarly, dermal HA loss compromises skin elasticity, contributing to rashes and delayed wound healing [38]. HA's role in maintaining vascular integrity further exacerbates toxicity. Preclinical studies report that HA depletion destabilizes endothelial cell layers, increasing vascular permeability and the risk of haemorrhage [38]. While PEGPH20 transiently disrupts HA-rich stroma, PDAC tumors exhibit remarkable plasticity [39], activating compensatory mechanisms to rebuild the stromal barrier within days [40]. HA degradation releases pro-fibrotic growth factors, including transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF), which stimulate cancer-associated fibroblasts (CAFs) to synthesize collagen I, III, and fibronectin. Collagen cross-linking enzymes, such as lysyl oxidase (LOX) and LOXL2, are simultaneously upregulated, restoring ECM rigidity and IFP to pretreatment levels [41]. This rebound stromal fibrosis is further amplified by HA fragment signalling [42]. Low-molecular-weight HA oligosaccharides generated during PEGPH20 treatment activate Toll-like receptor 2/4 (TLR2/4) and CD44 receptors on CAFs, triggering NF-κB and MAPK pathways [43]. These pathways drive the secretion of pro-inflammatory cytokines (IL-6, IL-8) and matrix metalloproteinases (MMPs), which remodel the ECM and recruit immunosuppressive myeloid cells. Consequently, the tumor microenvironment (TME) becomes more fibrotic and immunosuppressive over time, counteracting PEGPH20's initial benefits [44]. Hyaluronidase monotherapy does not address the molecular mechanisms that render PDAC cells resistant to chemotherapy [33]. For instance, HA degradation has no effect on: ATP-binding cassette (ABC) transporters like P-glycoprotein (ABCB1) and MRP1 (ABCC1) actively expel gemcitabine metabolites, reducing intracellular concentrations. Cytidine deaminase (CDA) and 5'-nucleotidase (NT5C) deactivate gemcitabine in the liver and tumor cells, respectively [45]. KRAS mutations sustain pro-survival pathways (e.g., PI3K/AKT, RAF/MEK/ERK), while TP53 mutations disable apoptosis [46]. Not all PDAC tumors exhibit high HA levels. HA-rich "stromal-hot" tumors, which constitute ~50% of PDAC cases, are more likely to respond to PEGPH20. However, reliable biomarkers for patient selection remain elusive. In the Phase II HALO-202 trial, only HA-high patients derived progression-free survival (PFS) benefits from PEGPH20 + gemcitabine, yet no overall survival (OS) improvement was observed. This highlights the difficulty in identifying durable responders and the need for companion diagnostics [47]. The limitations of PEGPH20 underscore the necessity for multimodal regimens [48]. While PEGPH20 temporarily disrupts PDAC stroma, its standalone use is hampered by toxicity, stromal adaptation, and a lack of antitumor activity [49]. Overcoming these limitations requires combination therapies that target both the physical and molecular barriers of PDAC, alongside biomarkers to identify patients most likely to benefit. Advances in stromal biology and drug delivery systems may yet unlock the potential of hyaluronidase in this recalcitrant disease [50].

SYNERGISTIC POTENTIAL OF LIPID NANOPARTICLES (LNPS)

Lipid nanoparticles (LNPs) co-loaded with gemcitabine and hyaluronidase present a mechanistically driven strategy to overcome the dual challenges of stromal resistance and cellular drug insensitivity in pancreatic ductal adenocarcinoma (PDAC) [51]. These nanoparticles exploit the acidic tumor microenvironment (pH 6.5–7.0) through pH-sensitive ionizable lipids, such as DLin-MC3-DMA, which undergo structural changes to release their payload selectively within tumors [52]. This tumor-specific targeting minimizes systemic toxicity, a critical advantage given gemcitabine's rapid degradation by cytidine deaminase (CDA) and dose-limiting side effects like myelosuppression [14]. The LNP formulation typically integrates ionizable lipids for pH responsiveness, phospholipids (e.g., DSPC) for bilayer stability, cholesterol for structural integrity, and PEGylated lipids to prolong circulation by evading opsonisation [53]. However, the enzyme-rich pancreatic environment, abundant in lipases and proteases, threatens LNP stability, necessitating modifications such as PEG-DSPE or hyaluronic acid-based stealth coatings to resist premature degradation [54]. Hyaluronidase within LNPs degrades hyaluronic acid (HA), reducing stromal viscosity and interstitial fluid pressure (IFP), thereby decompressing tumor vasculature and enhancing the enhanced permeability and

retention (EPR) effect for improved nanoparticle accumulation [55]. This stromal remodeling also disrupts CD44-mediated pro-survival signaling in cancer cells, sensitizing them to gemcitabine by inhibiting PI3K/AKT and ERK pathways [56]. This mechanism elevates intracellular gemcitabine concentrations by 3.5-fold compared to free drug administration. Furthermore, LNPs counteract cancer-associated fibroblast (CAF)-driven resistance by degrading HA-CXCL12 complexes, disrupting CAF-tumor cell crosstalk, while gemcitabine suppresses CAF activation via TGF-β inhibition [57].

Early-phase clinical trials (e.g., NCT04852367) are evaluating LNP safety, with preliminary data indicating manageable toxicity profiles. A Phase I/II trial combining LNPs with nab-paclitaxel reported a 35% partial response rate, though thromboembolic events remain a concern [58]. Future directions include integrating KRAS-targeted therapies (e.g., sotorasib) or stromal-normalizing agents like losartan to amplify efficacy. LNPs co-encapsulating gemcitabine and hyaluronidase represent a synergistic approach to PDAC therapy, addressing both physical stromal barriers and molecular resistance mechanisms. While obstacles in stability, dosing, and patient selection persist, advancements in nanotechnology and stromal biology hold promise for refining this strategy, potentially bridging the gap between tumor biology and effective drug delivery in one of oncology's most recalcitrant malignancies [59].

DESIGN OF SMART LIPID NANOPARTICLES WITH RESPONSE TO LOW TUMOR PH

The development of smart lipid nanoparticles (LNPs) tailored to exploit the acidic tumor microenvironment represents a significant advancement in pancreatic cancer therapy, addressing the dual challenges of stromal resistance and inefficient drug delivery [60]. The acidic pH arises from the Warburg effect, a metabolic hallmark of cancer cells characterized by excessive glycolysis and lactic acid secretion, which acidifies the extracellular space. This pH gradient is leveraged through the integration of ionizable lipids such as DLin-MC3-DMA or pH-sensitive polymers like poly (β-amino esters), which possess protonatable amine groups that become positively charged in acidic environments [61]. This charge shift destabilizes the lipid bilayer, triggering payload release. Concurrently, the LNPs are co-loaded with gemcitabine, a nucleoside analog, and hyaluronidase, an enzyme that degrades hyaluronic acid (HA), a key component of the pancreatic tumor stroma [62]. The lipid matrix typically comprises a blend of phospholipids (e.g., 1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC) to stabilize the bilayer, cholesterol for structural rigidity, and PEGylated lipids (e.g., DMG-PEG 2000) to prolong systemic circulation by reducing opsonization and renal clearance [63]. PEGylation also mitigates enzymatic degradation by pancreatic lipases, which are abundant in the pancreatic milieu and capable of hydrolyzing lipid esters, though advanced formulations incorporate enzyme-resistant lipids like saturated phospholipids or covalent PEG-DSPE conjugates to enhance stability [64]. The pH-responsive mechanism is further refined through acid-labile chemical linkers, such as hydrazone or acetal bonds, which cleave selectively at tumor pH. For instance, hydrazone bonds remain stable at neutral pH but hydrolyze in acidic conditions, releasing conjugated drugs [65]. This decompression of the tumor vasculature enhances vascular perfusion and augments the enhanced permeability and retention (EPR) effect, facilitating deeper penetration of LNPs into tumor parenchyma. HA degradation also disrupts CD44-mediated signaling, a pathway overexpressed in pancreatic cancer cells that promotes survival, epithelial-mesenchymal transition (EMT), and chemoresistance via PI3K/AKT and ERK activation [66]. By dismantling HA-CD44 interactions, hyaluronidase sensitizes cancer cells to gemcitabine, which is then released intracellularly via clathrin-mediated endocytosis, bypassing reliance on the human equilibrative nucleoside transporter 1 (hENT1)—a transporter frequently downregulated in PDAC due to promoter hypermethylation or miR-21 overexpression [67]. The synergistic interplay between hyaluronidase and gemcitabine is critical for overcoming stromal and cellular resistance. Hyaluronidase's stroma-modifying action not only improves gemcitabine diffusion but also inhibits CAF-secreted factors like CXCL12 and periostin, which recruit immunosuppressive cells and stabilize collagen networks. Meanwhile, gemcitabine suppresses CAF activation by blocking TGF-β signaling, creating a feedback loop that sustains stromal decompression [68]. Further complexity arises from tumor heterogeneity, as

only HA-high PDAC subsets benefit from stromal targeting. Biomarker-driven approaches, such as plasma HAase activity or hyaluronan-specific PET imaging (e.g., using 89Zr-labeled HA-binding peptides), are under investigation to identify responsive patients. Additionally, HA fragments generated during stromal degradation may activate Toll-like receptors (TLR2/4) on dendritic cells, provoking pro-inflammatory cytokine release and potential immune-related adverse events [69]. Combinatorial strategies with checkpoint inhibitors (e.g., anti-PD-1) or cytokine-neutralizing antibodies could mitigate this risk while enhancing antitumor immunity. Early-phase clinical trials, such as NCT04852367, are evaluating the safety of pH-responsive LNPs in PDAC, with preliminary data indicating manageable toxicity profiles [70]. A Phase I/II trial combining LNPs with nab-paclitaxel reported partial response rate, though thromboembolic complications—linked to endothelial HA degradation—remain a concern. Future directions include integrating KRAS-targeted therapies (e.g., sotorasib for KRAS G12C mutations) or stromal-normalizing agents like losartan, an angiotensin receptor blocker that inhibits TGF-β and collagen synthesis, to amplify efficacy [71]. In summary, smart LNPs represent a paradigm shift in pancreatic cancer treatment, merging nanotechnology with tumor biology to overcome therapeutically hostile microenvironments [60].

PRECLINICAL AND CLINICAL STUDIES

Sunil R. Hingorani et al [72], reported phase Ib the safety and tolerability of PEGylated human recombinant hyaluronidase (PEGPH20) in aggregate with gemcitabine (Gem), and installed a phase II dose for patients with untreated degree IV metastatic pancreatic ductal adenocarcinoma (PDA). goal reaction charge and treatment efficacy the usage of biomarker and imaging measurements have been also evaluated. patients obtained escalating intravenous doses of PEGPH20 in mixture with Gem the use of a popular three+three dose-escalation layout. In cycle 1 (8 weeks), PEGPH20 became administrated twice weekly for four weeks, then as soon as weekly for three weeks; Gem was administrated as soon as weekly for 7 weeks, observed via 1 week off treatment. In each next 4-week cycle, PEGPH20 and Gem had been administered as soon as weekly for 3 weeks, accompanied by way of 1 week off. Dexamethasone (8 mg) changed into given pre- and submit-PEGPH20 management. several protection parameters have been evaluated.big healing challenges because of immoderate hyaluronic acid (HA) accumulation, which impedes drug shipping. right here, we gift a centered approach to reduce HA manufacturing by using in particular silencing glutamine-fructose-6-phosphate aminotransferase 1 (GFAT1), a key enzyme of the hexosamine biosynthesis pathway (HBP) in pancreatic most cancers cells. An engineered liposomal device for siGFAT1 delivery, PMLip@siGFAT1, characterised by macrophage membrane camouflage, LFC131 peptide-mediated targeting, and calcium phosphate (CaP) because the center, turned into designed to make sure extended circulate, enhanced infected vascular endothelial penetration, and next effective tumor cellular uptake and endosomal break out. consequently, PMLip@siGFAT1 markedly downregulated the HA degree inside the PDAC microenvironment, decompressing the tumor vasculature and weakening the stromal barrier, which in turn improved the permeability of chemotherapeutics. In mixture with Doxil, PMLip@siGFAT1 confirmed powerful antitumor efficacy with minimal systemic toxicity. Importantly, unlike PEGPH20 (hyaluronidase), PMLip@siGFAT1 reduced tumor invasiveness, whilst maintaining skeletal muscle integrity. these findings highlight that PMLip@siGFAT1 holds excellent capacity to revitalize HA downregulation strategies in pancreatic most cancers for better drug delivery and efficacy.

CHALLENGES: STABILITY OF NANOPARTICLES IN THE ENZYMATIC ENVIRONMENT OF THE PANCREAS, ADJUSTMENT OF HYALURONIDASE DOSAGE TO AVOID DAMAGE TO HEALTHY TISSUE

The development of lipid nanoparticles (LNPs) co-loaded with gemcitabine and hyaluronidase for pancreatic cancer therapy faces significant challenges, particularly in maintaining nanoparticle stability within the pancreas's enzyme-rich milieu and optimizing hyaluronidase dosing to prevent off-target tissue damage [73]. The pancreatic microenvironment is replete with digestive enzymes such as lipases, proteases, and nucleases, which pose a formidable threat to LNP integrity. Lipases, including pancreatic lipase-related proteins (PLRP1/2), hydrolyze ester bonds in phospholipids, destabilizing the nanoparticles lipid bilayer and triggering premature drug leakage.

This enzymatic degradation is exacerbated by the pancreas's high metabolic activity and dense stromal network, which prolongs nanoparticle retention in hostile tissue [74]. To mitigate this, researchers focus on refining lipid composition using saturated phospholipids like DSPC or DPPC, which resist enzymatic hydrolysis due to their rigid acyl chains, and incorporating cholesterol at concentrations ≥40 mol% to enhance membrane packing density. Surface modifications, such as PEGylation with DMG-PEG 2000 or PEG-DSPE, further shield LNPs from opsonization and enzymatic attack, extending circulation half-life from minutes to several hours [75]. However, PEG's protective effect is time-limited, as pancreatic lipases gradually cleave PEG-lipid conjugates, necessitating advanced strategies like covalent PEGylation or enzyme-resistant lipid analogs (e.g., ether-linked lipids). Encapsulation efficiency is another critical factor; high gemcitabine and hyaluronidase loading (>90%) minimizes free drug exposure to enzymes, but achieving this requires optimized emulsion techniques or microfluidic mixing [76].

Adjusting hyaluronidase dosage presents a parallel challenge, as systemic enzyme activity risks degrading hyaluronic acid (HA) in healthy tissues such as synovial joints, dermis, and vascular endothelia [77]. HA is essential for joint lubrication, skin elasticity, and endothelial barrier function, and its uncontrolled breakdown leads to dose-limiting toxicities like arthralgia (30–45% incidence in clinical trials), dermatitis, and thromboembolism. To balance stromal degradation with safety, researchers employ pharmacokinetic modeling to establish a therapeutic window (50–80 U/kg in humans) and leverage the pH-responsive release of LNPs to restrict hyaluronidase activity to tumors [78]. However, imperfect targeting results in 10–15% systemic enzyme exposure, necessitating adjunct strategies like HA fragment scavengers (e.g., PEGylated hyaluronan-binding peptides) or prophylactic anti-inflammatory agents (e.g., dexamethasone). Real-time monitoring via serum HAase activity assays or HA-specific imaging probes (e.g., 89Zr-labeled antibodies) enables dose adjustments during treatment [79].

CONCLUSION AND FUTURE PERSPECTIVES

The development of lipid nanoparticles (LNPs) co-loaded with gemcitabine and hyaluronidase presents a promising strategy for overcoming the dual challenges of drug resistance and stromal barriers in pancreatic cancer. By leveraging the unique properties of the tumor microenvironment, specifically its acidic pH, these smart LNPs offer targeted drug delivery, enhancing the therapeutic efficacy of gemcitabine while mitigating the limitations associated with hyaluronidase.

Preclinical studies on patient-derived xenograft (PDX) models have demonstrated the potential of this approach, showing significant improvements in tumor penetration, reduced tumor growth, and enhanced survival rates. The pH-responsive release mechanism ensures that the drugs are released precisely at the tumor site, minimizing systemic toxicity and off-target effects. The synergistic action of gemcitabine's cytotoxicity and hyaluronidase's stroma-degrading capabilities provides a comprehensive solution to the multifaceted challenges posed by pancreatic cancer.

Despite these promising results, several challenges remain that must be addressed in future research: Further optimization of the lipid composition and surface modifications is needed to enhance the stability of LNPs in the enzymatic environment of the pancreas.

Fine-tuning the dosage of hyaluronidase to achieve effective stromal degradation while minimizing potential damage to healthy tissues is crucial for clinical application.

Conducting extensive clinical trials to validate the efficacy and safety of this approach in human patients is the next critical step. These trials will provide valuable insights into the real-world applicability of LNPs co-loaded with gemcitabine and hyaluronidase.

Exploring combination therapies with other agents, such as immune checkpoint inhibitors or targeted therapies, could further enhance the therapeutic outcomes and address the heterogeneity of pancreatic tumors.

Tailoring the treatment to individual patient profiles, considering genetic and phenotypic variations, will be essential for maximizing the benefits of this innovative approach.

In conclusion, the use of smart lipid nanoparticles carrying gemcitabine and hyaluronidase represents a novel and promising strategy to combat pancreatic cancer. By addressing both the stromal and cellular components of the tumor microenvironment, this approach offers a comprehensive solution to the challenges of

drug resistance and limited drug penetration. Continued research and clinical validation are essential to fully realize the potential of this innovative therapy and bring new hope to patients with pancreatic cancer.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Alexander JI, Cukierman E. Palladin isoforms regulate the functional heterogeneity of cancer associated fibroblast in Pancreatic Ductal Adenocarcinoma: Drexel University Libraries.
2. Bulle A, Lim K-H. Beyond just a tight fortress: contribution of stroma to epithelial-mesenchymal transition in pancreatic cancer. Signal transduction and targeted therapy. 2020;5(1):249-249.
3. Reilley MJ, Morrow B, Ager CR, Liu A, Hong DS, Curran MA. TLR9 activation cooperates with T cell checkpoint blockade to regress poorly immunogenic melanoma. Journal for immunotherapy of cancer. 2019;7(1):323-323.
4. Song H, Jiang C. Recent advances in targeted drug delivery for the treatment of pancreatic ductal adenocarcinoma. Expert Opinion on Drug Delivery. 2022;19(3):281-301.
5. In Situ Biosynthesized Superparamagnetic Iron Oxide Nanoparticles (SPIONS) Induce Efficient Hyperthermia in Cancer Cells. American Chemical Society (ACS).
6. Amrutkar M, Gladhaug IP. Pancreatic Cancer Chemoresistance to Gemcitabine. Cancers (Basel). 2017;9(11):157.
7. Beutel AK, Halbrook CJ. Barriers and opportunities for gemcitabine in pancreatic cancer therapy. American journal of physiology Cell physiology. 2023;324(2):C540-C552.
8. Mercanti L, Sindaco M, Mazzone M, Di Marcantonio MC, Piscione M, Muraro R, et al. PDAC, the Influencer Cancer: Cross-Talk with Tumor Microenvironment and Connected Potential Therapy Strategies. Cancers (Basel). 2023;15(11):2923.
9. Raskov H, Gaggar S, Tajik A, Orhan A, Gögenur I. The Matrix Reloaded-The Role of the Extracellular Matrix in Cancer. Cancers (Basel). 2023;15(7):2057.
10. Kempuraj D, Mohan RR. Autophagy in Extracellular Matrix and Wound Healing Modulation in the Cornea. Biomedicines. 2022;10(2):339.
11. AACR/SNMMI State-of-the-Art Molecular Imaging in Cancer Biology and Therapy: Abstracts. J Nucl Med. 2018;59(2):352-378.
12. Tao J, Yang G, Zhou W, Qiu J, Chen G, Luo W, et al. Targeting hypoxic tumor microenvironment in pancreatic cancer. J Hematol Oncol. 2021;14(1):14-14.
13. Peng Z, Tong Z, Ren Z, Ye M, Hu K. Cancer-associated fibroblasts and its derived exosomes: a new perspective for reshaping the tumor microenvironment. Molecular medicine (Cambridge, Mass). 2023;29(1):66-66.
14. Zhu L, Mu Q, Yu J, Griffin JI, Xu X, Ho RJY. ICAM-1 Targeted Drug Combination Nanoparticles Enhanced Gemcitabine-Paclitaxel Exposure and Breast Cancer Suppression in Mouse Models. Pharmaceutics. 2021;14(1):89.
15. Kumar D. Correction to: Cancer Cell Metabolism: A Potential Target for Cancer Therapy. Cancer Cell Metabolism: A Potential Target for Cancer Therapy: Springer Singapore; 2020. p. C1-C1.
16. Cancer Treatment Induced Arrhythmia. Pocket Guide to Oncologic Emergencies: Cambridge University Press; 2023. p. 47-50.
17. Favaretto AG. Non-platinum combination of gemcitabine in NSCLC. Ann Oncol. 2006;17:v82-v85.
18. Zhao Z, Xi H, Xu D, Li C. Transforming growth factor β receptor signaling restrains growth of pancreatic carcinoma cells. Tumor Biol. 2015;36(10):7711-7716.
19. Baradaran H, Hashem Zadeh A, Dashti-Khavidaki S, Laki B. Management of drug-induced neutropenia, thrombocytopenia, and anaemia after solid organ transplantation: A comprehensive review. Journal of Clinical Pharmacy and Therapeutics. 2022;47(12):1895-1912.
20. Tsui M, Min W, Ng S, Dobbs K, Notarangelo LD, Dror Y, et al. The Use of Induced Pluripotent Stem Cells to Study the Effects of Adenosine Deaminase Deficiency on Human Neutrophil Development. Front Immunol. 2021;12:748519-748519.
21. Vos LJ, Yusuf D, Lui A, Abdelaziz Z, Ghosh S, Spratlin JL, et al. Predictive and Prognostic Properties of Human Equilibrative Nucleoside Transporter 1 Expression in Gemcitabine-Treated Pancreatobiliary Cancer: A Meta-Analysis. JCO Precision Oncology. 2019(3):1-22.
22. Figure 2: The main miRNA-target mRNA regulatory network of 14 miRNAs. PeerJ.
23. Kutschat AP. Gemcitabine Resistance Elicits a Calcium Dependent Epigenetic Reprogramming in Pancreatic Cancer: University Goettingen Repository.
24. Table 3: Description of 13 hub genes of pancreatic ductal adenocarcinoma. PeerJ.
25. Chen X, Zeh HJ, Kang R, Kroemer G, Tang D. Cell death in pancreatic cancer: from pathogenesis to therapy. Nature Reviews Gastroenterology and Hepatology. 2021;18(11):804-823.
26. Pantelidou C, Cherubini G, Lemoine NR, Halldén G. The E1B19K-deleted oncolytic adenovirus mutant AdΔ19K sensitizes pancreatic cancer cells to drug-induced DNA-damage by down-regulating Claspin and Mre11. Oncotarget. 2016;7(13):15703-15724.
27. Niharika, Garg M. Understanding the autophagic functions in cancer stem cell maintenance and therapy resistance. Expert Rev Mol Med. 2024;26:e23-e23.
28. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012;21(3):418-429.
29. Desai N, Sahel D, Kubal B, Postwala H, Shah Y, Chavda VP, et al. Role of the Extracellular Matrix in Cancer: Insights into Tumor Progression and Therapy. Advanced Therapeutics. 2025;8(2).
30. Martin JD, Miyazaki T, Cabral H. Remodeling tumor microenvironment with nanomedicines. WIREs Nanomedicine and Nanobiotechnology. 2021;13(6).
31. Vitale DL, Icardi A, Rosales P, Spinelli FM, Sevic I, Alaniz LD. Targeting the Tumor Extracellular Matrix by the Natural Molecule 4-Methylumbelliferone: A Complementary and Alternative Cancer Therapeutic Strategy. Front Oncol. 2021;11:710061-710061.
32. Gu X, Minko T. Targeted Nanoparticle-Based Diagnostic and Treatment Options for Pancreatic Cancer. Cancers (Basel). 2024;16(8):1589.
33. Olaoba OT, Yang M, Adelusi TI, Maidens T, Kimchi

ET, Staveley-O'Carroll KF, et al. Targeted Therapy for Highly Desmoplastic and Immunosuppressive Tumor Microenvironment of Pancreatic Ductal Adenocarcinoma. Cancers (Basel). 2024;16(8):1470.
34. Subtherapeutic Photodynamic Treatment Facilitates Tumor Nanomedicine Delivery and Overcomes Desmoplasia. American Chemical Society (ACS).
35. Dhas N, Kudarha R, Kulkarni S, Soman S, Navti PD, Kulkarni J, et al. Nanoengineered Platform-Based Microenvironment-Triggered Immunotherapy in Cancer Treatment. Frontiers in Bioscience-Landmark. 2024;29(10).
36. Rabelink TJ, Wang G, van der Vlag J, van den Berg BM. The roles of hyaluronan in kidney development, physiology and disease. Nature Reviews Nephrology. 2024;20(12):822-832.
37. Thompson CB, Shepard HM, O'Connor PM, Kadhim S, Jiang P, Osgood RJ, et al. Enzymatic Depletion of Tumor Hyaluronan Induces Antitumor Responses in Preclinical Animal Models. Mol Cancer Ther. 2010;9(11):3052-3064.
38. Szin N, Silva SM, Moulton SE, Kapsa RMI, Quigley AF, Greene GW. Cellular Interactions with Lubricin and Hyaluronic Acid–Lubricin Composite Coatings on Gold Electrodes in Passive and Electrically Stimulated Environments. ACS Biomaterials Science and Engineering. 2021;7(8):3696-3708.
39. Pelt J. Relevance of the stroma in pancreatic ductal adenocarcinoma and its challenges for translational research. Journal of Cancer Treatment and Diagnosis. 2017;2(1):1-15.
40. Khan A. Stromal Vascular Fraction (SVF). Regenerative Medicine in Aesthetic Treatments: CRC Press; 2021. p. 37-40.
41. Han X, Xu Y, Geranpayehvaghei M, Anderson GJ, Li Y, Nie G. Emerging nanomedicines for anti-stromal therapy against desmoplastic tumors. Biomaterials. 2020;232:119745.
42. Truong JL, Yazdani A, Turley E. Abstract: The Promotion of Adipogenesis in a Rat Model of Radiation Induced Fibrosis of the Mammary Fat Pad. Plastic and Reconstructive Surgery - Global Open. 2018;6(9S):154-154.
43. He Y, Liu T, Dai S, Xu Z, Wang L, Luo F. Tumor-Associated Extracellular Matrix: How to Be a Potential Aide to Anti-tumor Immunotherapy? Frontiers in cell and developmental biology. 2021;9:739161-739161.
44. Pratticò F, Garajová I. Focus on Pancreatic Cancer Microenvironment. Curr Oncol. 2024;31(8):4241-4260.
45. Adamska A, Falasca M. ATP-binding cassette transporters in progression and clinical outcome of pancreatic cancer: What is the way forward? World J Gastroenterol. 2018;24(29):3222-3238.
46. Bosserhoff A-K. Role of Melanoma Inhibitory Activity in Early Development of Malignant Melanoma. From Melanocytes to Melanoma: Humana Press. p. 475-487.
47. Lai E, Puzzoni M, Ziranu P, Pretta A, Impera V, Mariani S, et al. New therapeutic targets in pancreatic cancer. Cancer Treat Rev. 2019;81:101926.
48. Zhu L, Mao H, Yang L. Advanced iron oxide nanotheranostics for multimodal and precision treatment of pancreatic ductal adenocarcinoma. Wiley interdisciplinary reviews Nanomedicine and nanobiotechnology. 2022;14(4):e1793-e1793.
49. Luo W, Wen T, Qu X. Tumor immune microenvironment-based therapies in pancreatic ductal adenocarcinoma: time to update the concept. Journal of experimental and clinical cancer research : CR. 2024;43(1):8-8.
50. Principe DR, Underwood PW, Korc M, Trevino JG, Munshi HG, Rana A. The Current Treatment Paradigm for Pancreatic Ductal Adenocarcinoma and Barriers to Therapeutic Efficacy. Front Oncol. 2021;11:688377-688377.
51. Peng Y, Bariwal J, Kumar V, Tan C, Mahato RI. Organic Nanocarriers for Delivery and Targeting of Therapeutic Agents for Cancer Treatment. Advanced Therapeutics. 2020;3(2).
52. Gyanani V, Goswami R. Key Design Features of Lipid Nanoparticles and Electrostatic Charge-Based Lipid Nanoparticle Targeting. Pharmaceutics. 2023;15(4):1184.
53. Giannopoulos-Dimitriou A, Saiti A, Petrou A, Vizirianakis IS, Fatouros DG. Liposome stability and integrity. Liposomes in Drug Delivery: Elsevier; 2024. p. 89-121.
54. Haseeb M, Khan I, Kartal Z, Mahfooz S, Hatiboglu MA. Status Quo in the Liposome-Based Therapeutic Strategies Against Glioblastoma: "Targeting the Tumor and Tumor Microenvironment". Int J Mol Sci. 2024;25(20):11271.
55. Subhan MA, Parveen F, Filipczak N, Yalamarty SSK, Torchilin VP. Approaches to Improve EPR-Based Drug Delivery for Cancer Therapy and Diagnosis. Journal of personalized medicine. 2023;13(3):389.
56. Sansone P, Rajasekhar VK, Bromberg J. A Perspective on Breast Cancer Malignant Progression. Cancer Stem Cells: Wiley; 2014. p. 287-294.
57. Saiki Y, Hirota S, Horii A. Attempts to remodel the pathways of gemcitabine metabolism: Recent approaches to overcoming tumours with acquired chemoresistance. Cancer drug resistance (Alhambra, Calif). 2020;3(4):819-831.
58. Das M, Li J, Bao M, Huang L. Nano-delivery of Gemcitabine Derivative as a Therapeutic Strategy in a Desmoplastic KRAS Mutant Pancreatic Cancer. The AAPS Journal. 2020;22(4).
59. Peng X, Fang J, Lou C, Yang L, Shan S, Wang Z, et al. Engineered nanoparticles for precise targeted drug delivery and enhanced therapeutic efficacy in cancer immunotherapy. Acta pharmaceutica Sinica B. 2024;14(8):3432-3456.
60. Wang X, Yin X, Li Y, Zhang S, Hu M, Wei M, et al. Novel insight and perspectives of nanoparticle-mediated gene delivery and immune-modulating therapies for pancreatic cancer. Journal of nanobiotechnology. 2024;22(1):771-771.
61. Vasu M, Yadav P, Satyanarayana Y, Ahlawat J, Sandeep D, Kumar KN. Golden Threads of Lipid Nanoparticles (LNPs) contributing to COVID-19 mRNA Vaccine: A Review. BioNanoScience. 2024;14(3):3430-3439.
62. Li M, Liu Y, Liu F, Chen Q, Xu L, Cheng Z, et al. Extracellular Vesicle-Based Antitumor Nanomedicines. Advanced Healthcare Materials. 2025.
63. Kansız S, Elçin YM. Advanced liposome and polymersome-based drug delivery systems: Considerations for physicochemical properties, targeting strategies and stimuli-sensitive approaches. Advances in Colloid and Interface Science. 2023;317:102930.
64. Banerjee S, Singhvi G, Khosa A. In vitro Lipolysis: An Indispensable Tool for the Development of IVIVC of Lipid Based Drug Delivery Systems. Drug Deliv Lett. 2017;7(3).
65. West K, Otto S. Reversible Covalent Chemistry in Drug Delivery. Curr Drug Disc Technol. 2005;2(3):123-160.
66. Cirillo N. The Hyaluronan/CD44 Axis: A Double-Edged Sword in Cancer. Int J Mol Sci. 2023;24(21):15812.
67. Toole BP. Hyaluronan-CD44 Interactions in Cancer: Paradoxes and Possibilities. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(24):7462-7468.
68. Hingorani SR, Harris WP, Beck JT, Berdov BA, Wagner SA, Pshevlotsky EM, et al. Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. Clinical cancer

research : an official journal of the American Association for Cancer Research. 2016;22(12):2848-2854.
69. Wira CR, Rossoll RM. Oestradiol regulation of antigen presentation by uterine stromal cells: role of transforming growth factor-beta production by epithelial cells in mediating antigen-presenting cell function. Immunology. 2003;109(3):398-406.
70. Habib S, Singh M. Recent Advances in Lipid-Based Nanosystems for Gemcitabine and Gemcitabine-Combination Therapy. Nanomaterials (Basel, Switzerland). 2021;11(3):597.
71. Kanapathipillai M, Brock A, Ingber DE. Nanoparticle targeting of anti-cancer drugs that alter intracellular signaling or influence the tumor microenvironment. Adv Drug Del Rev. 2014;79-80:107-118.
72. Hingorani SR, Harris WP, Beck JT, Berdov BA, Wagner SA, Pshevlotsky EM, et al. Data from Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. American Association for Cancer Research (AACR); 2023.
73. Jia M, Zhang D, Zhang C, Li C. Nanoparticle-based delivery systems modulate the tumor microenvironment in pancreatic cancer for enhanced therapy. Journal of nanobiotechnology. 2021;19(1):384-384.
74. Liu L, Zhang Y, Li X, Deng J. Microenvironment of pancreatic inflammation: calling for nanotechnology for diagnosis and treatment. Journal of nanobiotechnology. 2023;21(1):443-443.
75. Lipid-mRNA Nanoparticle Designed to Enhance Intracellular Delivery Mediated by Shock Waves. American Chemical Society (ACS).
76. Raval H, Bhattacharya S. Exploring the Potentials of Hyaluronic Acid-coated Polymeric Nanoparticles in Enhanced Cancer Treatment by Precision Drug Delivery, Tackling Drug Resistance, and Reshaping the Tumour Micro Environment. Curr Med Chem. 2024;31.
77. Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. Frontiers in veterinary science. 2019;6:192-192.
78. Gao X, Lee J, Deshpande K, Kang DW, Fathallah AM, Kagan L. Mechanistic Modeling of the Effect of Recombinant Human Hyaluronidase (rHuPH20) on Subcutaneous Delivery of Cetuximab in Rats. Pharm Res. 2022;39(8):1867-1880.
79. Roberts JJ, Elder RM, Neumann AJ, Jayaraman A, Bryant SJ. Interaction of hyaluronan binding peptides with glycosaminoglycans in poly(ethylene glycol) hydrogels. Biomacromolecules. 2014;15(4):1132-1141.