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Review

Nanomedicine and epigenetics: New alliances to increase the odds in pancreatic cancer survival

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancers worldwide, primarily due to its robust desmoplastic stroma and immunosuppressive tumor microenvironment (TME), which facilitate tumor progression and metastasis. In addition, fibrous tissue leads to sparse vasculature, high interstitial fluid pressure, and hypoxia, thereby hindering effective systemic drug delivery and immune cell infiltration. Thus, remodeling the TME to enhance tumor perfusion, increase drug retention, and reverse immunosuppression has become a key therapeutic strategy. In recent years, targeting epigenetic pathways has emerged as a promising approach to overcome tumor immunosuppression and cancer progression. Moreover, the progress in nanotechnology has provided new opportunities for enhancing the efficacy of conventional and epigenetic drugs. Nano-based drug delivery systems (NDDSs) offer several advantages, including improved drug pharmacokinetics, enhanced tumor penetration, and reduced systemic toxicity. Smart NDDSs enable precise targeting of stromal components and augment the effectiveness of immunotherapy through multiple drug delivery options. This review offers an overview of the latest nano-based approaches developed to achieve superior therapeutic efficacy and overcome drug resistance. We specifically focus on the TME and epigenetic-targeted therapies in the context of PDAC, discussing the advantages and limitations of current strategies while highlighting promising new developments. By emphasizing the immense potential of NDDSs in improving therapeutic outcomes in PDAC, our review paves the way for future research in this rapidly evolving field.

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1. Introduction

Although pancreatic ductal adenocarcinoma (PDAC) is a fairly infrequent cancer type, it is one of the deadliest, currently ranking 7th in cancer-related deaths worldwide and predicted to become the 2nd leading cause by 2030 in the US [1,2]. Moreover, despite extensive research, its incidence continues to rise steadily every year [3]. Furthermore, PDAC has a dismal prognosis, with a 5-year survival rate of just 12% [4], thus, early diagnosis is crucial for improving the prognosis. The risk of developing PDAC is influenced by non-modifiable risk factors such as age, ethnicity, type II diabetes, family history of cancer and genetic cancer syndromes, chronic pancreatitis, and intraductal papillary mucinous neoplasms. Other modifiable lifestyle-related factors include cigarette smoking, physical inactivity, obesity, and alcohol intake. Given the limited and mainly chemotherapy-based treatment options available, their suboptimal therapeutic efficacy, and the persistently poor outcomes observed in PDAC patients due to advanced disease, an urgent need for innovative and novel therapeutic approaches exists.

Therefore, this review aims to investigate how the synergistic combination of epigenetic drugs and nano-based approaches can modulate the tumor microenvironment (TME) to improve PDAC outcomes. Simultaneously, various aspects of PDAC pathogenesis, genetic and epigenetic heterogeneity, and the potential of nanotechnology to enhance PDAC treatment efficacy are discussed. The use of nanotechnology to enhance drug tumor penetration, remodeling of the stroma, and modulation of the immunosuppressive microenvironment, including the application of epigenetic therapy, is reviewed in detail. This review also includes information about ongoing clinical trials that investigate the efficacy of Food and Drug Administration (FDA)-approved epigenetic drugs, either alone or in combination with other therapies, as well as promising nanocarriers for treating pancreatic cancer. The novelty of the current review is its critical overview of in vitro and in vivo models used for PDAC research. The advantages and limitations of these models are highlighted, and strategies to harmonize regulatory practices for the risk assessment of nano-based drugs are also discussed. This perspective on model systems and regulatory considerations adds depth to the review, addressing important aspects for the successful translation of nanotechnology-based therapies in the clinic.

To ensure an in-depth and thorough review of the topic, the article search strategy was conducted using PubMed and Google Scholar databases, focusing on peer-reviewed journal articles published over a 10-year period between 2013 and 2023. By encompassing the most recent literature in this fast-moving field, this review provides up-to-date information on the subject matter.

2. Molecular background and current therapeutic approaches

The development of PDAC is characterized by the transformation of normal pancreatic ductal or acinar cells into preinvasive precursor lesions known as pancreatic intraepithelial neoplasia (PanIN). Advanced PanINs are highly diverse and are associated with genetic instability and increased cell proliferation [5]. The accumulation of mutations, epigenetic alterations, and metabolic rearrangements drive the progression of PDAC. These changes result in the activation of multiple signaling pathways involved in the regulation of growth and proliferation, as well as alterations in the expression of several tumor-suppressor genes [6]. The most frequent somatic mutations in PDAC arise in the *KRAS* oncogene and are present in over 90% of tumors, affecting cell proliferation, differentiation, and survival [7]. KRAS also helps to shape the characteristic TME by modulating the release of various cytokines and growth factors [8]. For example, granulocyte-macrophage colony-stimulating factor secreted by tumor cells with $KRAS^{G12}$ mutations can mobilize $Gr1^{+}CD11b^{+}$ myeloid cells (i.e., macrophages and transiently differentiating monocytes) and $CD4^{+}Foxp3^{+}CD25^{+}$ regulatory T cells (Tregs) [9]. Other studies have shown that the presence of $KRAS^{G12D}$ mutation in PDAC aids in the recruitment of macrophages and immunosuppressive myeloid-derived suppressor cells (MDSCs) [10], as well as promoting NF-κB activation [11]. An elegant study by Ischenko et al., using a PDAC mouse model with CRISPR-mediated inactivating KRAS, showed that KRAS is a key mediator of the immunosuppressive PDAC TME [12]. Other somatic mutations found in over 50% of PDACs include inactivating mutations or epigenetic alterations in the tumor-suppressor genes *TP53*, *SMAD4*, and *CDKN2A* [13]. Interestingly, a 2021 study by Siolas et al. [14] showed that the gain-of-function $p53^{R172H}$ mutation drives the accumulation of neutrophils in PDAC, which plays an important role in resistance to immunotherapy.

Currently, the only potentially curative treatment for PDAC is surgical resection. Neoadjuvant and adjuvant approaches based on chemotherapy regimens such as FOLFIRINOX (folinic acid, 5-fluorouracil [5-FU], irinotecan, and oxaliplatin), gemcitabine (GEM) with nab-paclitaxel (Abraxane) (GEM-NAB) or capecitabine, with or without radiotherapy resulted in increased disease-free survival (DFS) and rate of pathologic complete remissions (pCR). However, most patients (79%) experienced disease recurrence or relapse even after aggressive treatment [15].

As a first-line therapy, FOLFIRINOX was effective in the PRODIGE/ACCORD11 trial, increasing the median overall survival (OS) by 4.2 months compared to GEM monotherapy. The MPACT trial compared GEM monotherapy with GEM-NAB. Combination treatment prolonged OS to 8.5 months, compared to 6.7 months, with GEM monotherapy [16]. FOLFIRINOX is considered generally more toxic than the GEM-NAB combination and therefore, its use is limited to patients with a performance status and favorable comorbidity profiles. Second-line therapy was only recently defined, with results stemming from the NAPOLI-1 trial, where nanoliposomal irinotecan (nal-IRI, Onivyde) combined with 5-FU increased OS by 2.1 months in comparison to 5-FU/leucovorin (LV) monotherapy [17].

So far, therapy with immune checkpoint inhibitors (ICIs) or in combination with chemotherapy has shown limited to no efficacy in treating PDAC [18]. Pembrolizumab (PD-1 inhibitor) is the only FDA-approved ICI in metastatic microsatellite instability-high (MSI-H) PDAC tumors. However, MSI-H status is present only in about 2% of patients [19]. Chimeric antigen receptor T cell (CAR-T)-based therapies have also shown little efficacy in PDAC, although several Phase I CAR-T cell clinical trials have already demonstrated safety, maximum tolerated doses, and some early signals of response to treatment in PDAC [20], most recently with Claudin18.2 CAR-T cells [21]. Interestingly, a recent study using T-cells engineered to target $KRAS^{G12D}$ for the regression of visceral metastases provided some hope that operational CAR-T cells can be clinically efficacious for PDAC [22]. However, controversy exists as to the real clinical applicability of anti-$KRAS^{G12D}$ CAR-T cells. Encouraging results were recently published by Rojas et al. [23] with an individualized adjuvant vaccine based on uridine mRNA–lipoplex nanoparticles (cevumeran) in combination with atezolizumab (an anti-PD-L1 immunotherapy) and modified mFOLFIRINOX (four-drug regimen). The Phase I clinical trial results revealed the capacity of autogene cevumeran to expand neoantigen-specific and functional $CD8^{+}$ T cells and demonstrated clinical advantage in patients with surgically resected PDAC (NCT04161755).

Loss of function of core genes responsible for homologous recombination repair (*BRCA1, BRCA2, PALB2*) was shown to predict sensitivity to platinum compounds and poly-ADP-ribose-polymerase inhibitors (PARPi). Based on results from the POLO trial, Olaparib is an FDA-approved maintenance therapy for germline *BRCA1*-positive PDAC patients who did not progress on previous platinum-based therapy [24]. However, maintenance treatment with Olaparib did not increase OS [25].

Until recently, *KRAS* was deemed "undruggable" [26]. Nevertheless, in recent years, novel irreversible and selective $KRAS^{G12C}$ inhibitors (e.g., Sotorasib and Adagrasib) have entered clinical trials (NCT05251038. NCT05634525, NCT03785249, NCT04975256, NCT04330664). PDAC

patients with $KRAS^{G12C}$ mutations showed modest activity with objective response rates ranging from 21.1% to 50%, respectively [27,28]. Since $KRAS^{G12C}$ mutations represent approximately 2% of all KRAS mutations in PDAC, other approaches have been evaluated, such as targeting KRAS signaling upstream and downstream. For example, Ruess et al. [29] and Frank et al. [30] used inhibitors of the downstream KRAS effectors MEK (Trametinib) or ERK (LY3214996), respectively, in combination with allosteric inhibitors of the ubiquitously expressed non-receptor protein tyrosine phosphatase SHP2, upstream of KRAS. These studies showed potent inhibition of $KRAS^{G12D}$ tumors in PDAC genetically engineered mouse models and patient-derived xenografts (PDXs). The SHP2/ERK inhibitor study results have led to a Phase I clinical trial (SHERPA; ClinicalTrials.gov: NCT04916236), enrolling patients with KRAS-mutant PDAC.

KRAS wild-type PDAC with higher rates of MSI, offers alternative treatment opportunities by targeting upstream and downstream components such as *EGFR, ALK, ROS1, NRG1, NTRK, RET, PI3K,* and *BRAF* [31]. In addition, Entrectinib, Larotrectinib (NTRK inhibitors), and Selpercatinib (RET inhibitor) were approved by the FDA as tumor-agnostic therapies. However, this only applies to around 5% of PDAC cases.

3. Epigenetic mechanisms shaping the tumor microenvironment

The TME, consisting of acellular and cellular components, contributes significantly to PDAC aggressiveness [32,33]. The robust desmoplastic reaction and extensive immunosuppressive environment associated with the PDAC TME [34,35] facilitates tumor cell proliferation, metastasis, and immune response evasion [36]. In addition, excessive extracellular matrix (ECM), accounting for 80–90% of the tumor volume, creates a physical barrier that hinders drug delivery and limits the supply of oxygen and nutrients. Moreover, collapsing tumor vasculature limits immune cell access, creating an immunologically cold environment [37]. Besides tumor cells, the TME is composed of pancreatic stellate cells (PSCs), cancer-associated fibroblasts (CAFs), and various non-tumorigenic hematopoietic cells, including MDSCs, tumor-associated macrophages (TAMs), tumor endothelial cells, and Tregs [34]. CAFs originate from quiescent fibroblasts after activation by various external signals such as growth factors (TGF-β), cytokines (TNF-α, interleukins – IL-1β, IL-6), and signaling molecules (sonic hedgehog, SHH). Activated CAFs secrete different extracellular components, such as collagen, hyaluronic acid (HA), fibronectin, chemokines, cytokines, matrix metalloproteinases (MMPs), and growth factors. The description of specific strategies aimed at targeting the TME and its cellular and non-cellular constituents is beyond the scope of this review but have been reviewed in several other publications [38–40].

Another field that has recently gained substantial attention is elucidating the role of individual epigenetic mechanisms in the regulation of the PDAC TME. Epigenetic changes refer to heritable modifications of DNA, histone, or chromatin structures that affect gene expression without changing the DNA sequence [41]. They are essential contributors to PDAC development, progression, and survival [42–44]. The complex and dynamic epigenetic landscape of PDAC plays a significant role in defining the molecular subtypes of PDAC. Epigenomics consistently distinguishes two major PDAC subtypes, basal-like, associated with a more mesenchymal expression profile, higher tumor grade, chemoresistance, and poor prognosis; and the classical subtype, comprised of an epithelial-like gene signature, lower tumor grade, and better prognosis [45]. Epigenetic mechanisms also play a pivotal role in controlling the dynamic plasticity of PDAC stroma components. DNA methylation and chromatin remodeling have been critically involved in cancer immunopathology, including tumor antigen presentation, T-cell infiltration, and disruption of the immunosuppressive state [46]. Overexpression of DNA methyltransferase 1 (DNMT1), the enzyme responsible for transferring methyl groups during DNA methylation, was observed in approximately 80% of PDAC cell lines [47]. DNMT1 inhibition is associated with increased tumor immunogenicity and immune recognition via the upregulation of surface MHC-I expression and release of IFNγ by tumor-specific $CD8^+$ T cells [48]. The upregulation of most MHC-I-coding genes was also induced by the ablation of the histone demethylase LSD1 [49]. Histone methyltransferases (HMTs) add methyl groups to specific residues of histone proteins. For example, the epigenetic regulator enhancer of zeste homolog 2 (EZH2) represses gene expression by catalyzing lysine 27 trimethylation (H3K27me3). EZH2 and DNMT1 were shown to repress the tumor production of T helper 1 (TH1)-type chemokines and determined effector T-cell trafficking to the TME [50]. Moreover, EZH2 and DNMT1 were negatively associated with tumor-infiltrating $CD8^+$ T cells and patient outcomes. EZH2 plays a critical role in Treg cell differentiation and maintenance of Treg cell identity, and Treg-cell-specific deletion of EZH2 results in spontaneous autoimmunity [51]. Consistently, either pharmacological or genetic disruption of EZH2 activity in Treg cells leads to the acquisition of pro-inflammatory gene signatures, with increased $CD4^+$ and $CD8^+$ T cell recruitment into the TME to promote antitumor immunity [52]. However, administration of the EZH2 inhibitor GSK126 resulted in increased MDSC accumulation and fewer $CD4^+$ and $IFN\gamma^+CD8^+$ T cells within the TME, which is consistent with the finding that inhibition of EZH2 activity promotes MDSC generation from hematopoietic progenitor cells in vitro [53].

Cancer cells have also been shown to downregulate immune sensing via epigenetic silencing of antitumor cytokines, chemokines, and induction of the immune checkpoint PD-L1 [54]. Inhibition of EZH2 was shown to increase the production of the inflammatory chemokine CXCL10, while inhibition of DNMT1 promoted both CXCL9 and CXCL10 mRNA and protein expression. Furthermore, targeted inhibition of EZH2 and DNMT1 triggered enhanced effector T-cell trafficking into the TME and delayed tumor growth [50]. Tumor-cell-derived CCL5 is critical for efficient T-cell infiltration into the TME and loss of CCL5 expression in human tumors was associated with DNA hypermethylation [55]. The DNMT1 inhibitor 5-azacytidine (AZA) was shown to reduce PDAC progression by influencing global DNA methylation in PDAC epithelial cells and CAFs [56]. In addition, DNMT1 inhibition in immunocompetent PDAC models enhanced $CD4^+$ and $CD8^+$ T-cell infiltration and caused significant tumor regression [57]. On the other hand, DNMT1 depletion led to increased hyaluronic acid (HA) production in vitro [58]. Recently Espient et al. [59] demonstrated that tumors with low global DNA methylation are characterized by protumorigenic reprogramming. In a syngeneic orthotopic PdxCre;LSLKrasG12D;LSL-Trp53R172H (KPC) PDAC mouse model, systemic administration of the pan-HDAC inhibitor SAHA (suberoylanilide hydroxamic acid, also known as Vorinostat), suppressed tumor growth as compared to untreated controls. However, the resulting tumors lacked the dense stroma typically observed in PDAC and were entirely composed of tumor cells. Furthermore, HDAC inhibitor-treated fibroblasts exhibited enhanced biological aggressiveness, as evidenced by increased secretion of pro-inflammatory tumor-supportive cytokines and chemokines [60]. These results highlight the complexities associated with epigenetic targeting strategies. The bromodomain and extraterminal (BET) proteins are critical cofactors that promote enhancer activity and play a significant role in PDAC progression [45,61]. They regulate TGF-β, SHH signaling, and as a consequence, CAF activity [62]. Importantly different BET inhibitors have distinct specificities towards BET protein family members and, thus, different consequences. They seem particularly efficient when combined with standard chemotherapy or other epigenetic regulators [63]. EZH2 is a chromatin regulatory protein directing TME-reprogramming in PDAC. Its deficiency in an Ezh2-deficient Kras mutant transgenic mouse model led to increased collagen deposition and promoted carcinogenesis [64,65]. Importantly, in this model, Ezh2 ablation occurred in epithelial and not in stromal cells, highlighting a communication between these cellular components. Inhibition of MLL1 methyltransferase, responsible for installing H3K4me3 histone marks, has been reported to prevent PD-L1 expression and immune cell evasion

[66]. The combination of anti-PD-1 or anti-CTLA-4 inhibitors with HDAC inhibition led to a significant abundance of cytotoxic T cells by decreasing the activity of MDSCs in the TME [67]. A similar approach based on HDAC/DNMT inhibition combined with chemotherapy, followed by PD-L1 blockade is undergoing clinical validation (NCT04257448). As epigenetic TME reprogramming focused on sensitizing tumors to immunotherapy has rarely been studied in PDAC, this approach represents a novel strategy for disrupting the immunosuppressive state of the PDAC TME. Current findings suggest that epigenetic drugs may help promote tumor cell immunogenicity or reeducate TAMs, MDSCs, or Tregs to support T cell effector functions (Fig. 1).

3.1. Epigenetic drugs: application challenges

Several epigenetic drugs have already been approved for the treatment of hematological malignancies by the FDA and the European Medicines Agency (EMA), including AZA, decitabine (DAC), SAHA, Romidepsin, Panobinostat, and Tazemetostat. Currently, several of them are undergoing testing as monotherapies or in combination with other therapeutic modalities for the treatment of PDAC and other solid tumors (Table 1).

These epigenetic drugs focus on the dynamic plasticity between PDAC molecular subtypes, aiming to switch tumors from an aggressive basal-like subtype into a less aggressive classical subtype [68]. Nevertheless, the clinical effectiveness of epigenetic drugs is hindered by several challenges, including poor solubility, stability, bioavailability, or non-specific distribution, making it difficult to attain therapeutic concentrations at the tumor site [69,70]. In addition, targeting epigenetic modifications in both cancerous and non-cancerous cells leads to off-target effects and potential toxicity. Global and unpredictable consequences can be mediated by targeting diverse HDAC proteins with pan-HDAC inhibitors. Tumor cells may develop resistance to epigenetic drugs through various mechanisms, such as the upregulation of drug efflux pumps or alterations in the epigenetic landscape. Another obstacle affecting the clinical effectiveness of epigenetic drugs is the physical barrier created by the dense ECM and collapsed vasculature, which can limit the penetration and efficacy of drugs. Additionally, PDAC, like other solid tumors, is highly heterogeneous, and epigenetic modifications can vary among tumor cells within the same patient, posing challenges to develop effective therapies that target all subpopulations of cancer cells. Furthermore, the lack of reliable biomarkers to predict which patients will benefit from epigenetic therapies presents a challenge in tailoring treatment strategies for individual patients. Thus, there is a need to develop novel and inventive approaches to overcome these obstacles and facilitate the clinical application of epigenetic drugs for treating PDAC and other solid tumors.

4. In the era of nanomedicine: nanoscale-drug delivery systems

Nanotechnology has revolutionized drug design by introducing innovative nanoscale-drug delivery systems (NDDSs) that allow further optimization and multi-functionalization through simple modifications. The advantage of NDDSs lies in their ability to simultaneously deliver hydrophilic and hydrophobic drugs, aiding tumor diagnosis and treatment (theranostics), and achieving controlled drug release. Encapsulating conventional drugs in nanoparticles (NPs) can overcome their poor water solubility, protect them from premature enzymatic degradation, reduce their adverse side effects, and increase their stability and pharmacokinetics. Improved pharmacokinetics and circulation in the body may lead to enhanced drug accumulation at the tumor site through passive targeting, known as the enhanced permeability and retention (EPR) effect. Additionally, the conjugation of specific ligands to NPs, can assist in active targeting and bypassing of biological barriers for delivering high drug concentrations to the target tissue. Controlled drug release from NPs can be triggered by pH, temperature, redox potential, and other stimuli. NDDSs administered systemically should have sizes ranging from 10 to 200 nm to avoid rapid elimination by the kidneys and subsequent entrapment by the spleen and liver. Biocompatibility, low toxicity, and immunotoxicity are essential aspects of biomedical nanomaterials, which undergo rigorous pre-clinical and clinical testing to ensure biosafety before FDA approval. The range of nano-based delivery systems, including liposomes, solid lipid NPs, polymer NPs, polymer micelles, dendrimers, nanoemulsions, and polymer-lipid hybrid NPs, is expanding, offering promising options for targeted drug delivery [69]. Nearly 100 nanomedicine products with excellent pharmacokinetic properties have already been approved by the FDA and EMA for

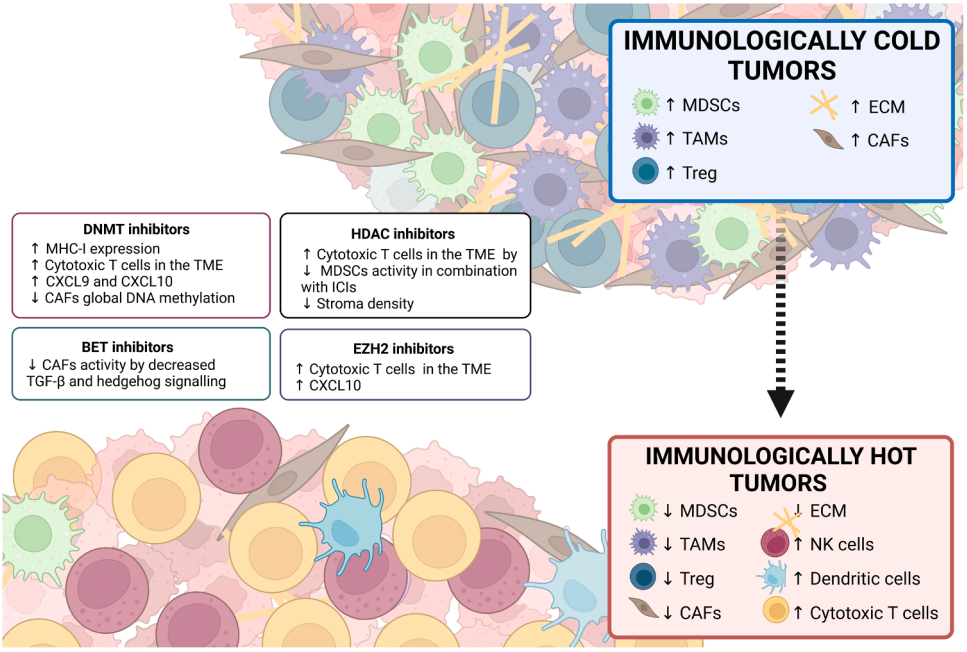

Fig. 1. Effect of epigenetic inhibitors on tumor microenvironment and immune cells. Abbreviations: BET, bromodomain and extra-terminal domain; CAF, cancer-associated fibroblasts; DNMT, DNA methyltransferase; ECM, extracellular matrix; HDAC, histone deacetylase; EZH2, enhancer of zeste homolog 2; ICIs, immune checkpoint inhibitors; MDSCs, myeloid-derived suppressor cells; NK cells, natural killer cells; TAMs, tumor-associated macrophages; Treg, regulatory T cells.

Table 1
Clinical trials testing the efficacy of epigenetic drugs alone or in combination with other therapies.

Identifier	Epigenetic drug	Conventional drug	Indication	Phase	Sponsor
DNA methylation inhibitors					
NCT03264404	Azacytidine	Pembrolizumab (Anti-PD1)	PDAC	Phase II	Ruth A. White, MD, Ph.D.
NCT03257761	Guadecitabine	Durvalumab (Anti-PDL1)	Bile duct adenocarcinoma, Gallbladder adenocarcinoma, Metastatic PDAC, Hepatocellular Ca	Phase I	University of Southern California
NCT05360264	Decitabine (Dacogen)	-	PDAC, Metastatic PDAC, Recurrent tumors expressing a KRAS-dependency signature	Phase II	Luca Cardone
NCT01845805	Azacitidine	Abraxane, gemcitabine	PDAC	Phase II	https://pubmed.ncbi.nlm.nih.gov/36463226/
HDAC inhibitors					
NCT01638533	Romidepsin	-	PC and other solid tumors, hematologic malignancies	Phase I	National Cancer Institute
NCT04705818	Tazemetostat (Tazverik) EZH2 inhibitor	Durvalumab	Advanced solid tumors, Advanced CRC, Advanced soft-tissue sarcoma, Advanced PDAC, Adult solid tumors	Phase II	Institut Bergonié
NCT03878524	Vorinostat	52 drugs (chemotherapy, small inhibitors, antibodies)	PDAC and other solid tumors, hematologic malignancies	Phase I	OHSU Knight Cancer Institute
NCT03878524	Panobinostat, Vorinostat	In combination with more than 50 drugs	PDAC and other solid tumors, hematologic malignancies	Phase I	OHSU Knight Cancer Institute
NCT05053971	Entinostat (does not have regulatory approval yet)	ZEN003694 (BET inhibitor)	PC, Lymphoma, Non-Hodgkin lymphoma	Phase I/II	National Cancer Institute
NCT03250273	Entinostat	In combination with Nivolumab	PDAC and Cholangiocarcinoma	Phase II	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
NCT02349867	Vorinostat	Gemcitabine, Sorafenib, Radiation Therapy	PDAC	Phase I	Virginia Commonwealth University
DNA methylation and HDAC inhibitors					
NCT04257448	Part 1: Azacitidine or Romidepsin or Azacitidine+ Romidepsin Part 2: Patients from Part 1	Abraxane/Gemcitabine, Durvalumab, Lenalidomide capsule	PDAC	Phase I/II	GWT-TUD GmbH

Abbreviations: PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal cancer; Ca, carcinoma; BET, bromodomain and extra-terminal domain

clinical use. Moreover, many other nanoformulations are being evaluated in clinical trials and preclinical studies [71]. By minimizing non-specific interactions, NDDSs enhance the therapeutic potential of nanomedicines [72].

4.1. Nano-based drug delivery systems: current application and testing

Progress in NDDS design and engineering offers new opportunities for more sophisticated strategies to enhance PDAC therapeutic efficacy and patient outcomes [73]. Several nanomedicines have been approved for the treatment of PDAC (Table 2).

The most commonly used nanomedicine in PDAC includes Abraxane™, an albumin-paclitaxel (PTX) conjugate authorized by the FDA in 2013, as a first-line treatment for metastatic PDAC in combination with GEM [74]. In addition, Onivyde™, a liposomal nanocarrier of irinotecan, gained FDA approval in 2015 as a second-line treatment option in combination with 5-FU and leucovorin in metastatic PDAC with poor response to GEM monotherapy [17]. Abraxane™ and Onivyde™ are currently used in many clinical trials evaluating their effectiveness in combination with conventional anticancer drugs to improve patient outcomes (Table 3).

Liposomes and lipid-based NPs represent the most widespread category of NDDSs in PDAC treatment [75], followed by polymeric NPs [76], protein-drug conjugate NPs [77], vaccines, especially mRNA vaccines [78], inorganic NPs [79], or extracellular vesicle (exosomes)-based NPs [80]. Some new NDDSs are currently under clinical evaluation (Table 4). In addition to therapeutic vaccines (NCT04853017) and antibody-drug conjugates (NCT04601285, NCT04175847, NCT03859752), some current clinical trials are evaluating nanocarriers encapsulating conventional drugs with improved pharmacokinetics as monotherapy or combined therapy (NCT04640480, NCT03537690, NCT03382340, NCT04852367) or

Table 2
Approved and marketed nano-based drugs for the treatment of pancreatic cancer.

Product name	Type of nanocarrier	Drug agent	Indication	Approval (Year)	Sponsor
Onivyde™	Liposome	Irinotecan	Metastatic PDAC, CRC	FDA (2015)	Merrimack
Abraxane™	Protein-drug conjugate	Paclitaxel	BC, NSCLC, metastatic PDAC	FDA (2005)	American Biosciencem, Inc.
Pazenir™	Protein-drug conjugate (a genericum)	Paclitaxel	Metastatic BC, Metastatic PDAC, NSCLC	EMA (2019)	Ratiopharm GmbH
NanoTherm®	Metallic nanoparticles	Fe_2O_3	Glioblastoma, prostate cancer and PC	EMA (2013)	Magforce
Nano/microparticle imaging agents					
SonoVue® (Ultrasound contrast agent)	Phospholipid stabilized microbubble	Sulphur hexafluoride	Ultrasound enhancement for: liver neoplasms, prostate cancer, BC, PDAC, or coronary/pulmonary disease	EMA (2001)	Bracco Imaging

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; BC, breast cancer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PC, pancreatic cancer; FDA, the US Food and Drug Administration; EMA, the European Medicines Agency

Table 3
Ongoing clinical trials involving Abraxane™ and Onivyde™ in combination with other therapeutic approaches for the treatment of pancreatic cancer.

Identifier	Combined therapy	Indication	Phase	Sponsor
NCT02340117	mFOLFIRINOX vs. Gemcitabine/**Abraxane™**	Metastatic PDAC	Phase II	Southwest Oncology Group
NCT01676259	**siG12D-LODER:** a novel, miniature bio-degradable polymeric matrix encompassing a novel small interfering RNA targeting KRAS G12D and all additional G12X mutations (G12C, G12V.) in combination with Gemcitabine+**Abraxanne™** or FOLFIRINOX/ mFOLFIRINOX	Locally advanced PDAC	Phase II	Silenseed Ltd
NCT03861702	**Onivyde™** in combination with the FOLFOX regimen	Locally advanced PDAC	Phase II	Nelson Yee
NCT04796948	**Onivyde™** in combination with the FOLFOX regiment	Advanced PDAC without prior systemic chemotherapy	Phase I	Jiangsu HengRui Medicine Co., Ltd.
NCT03483038	**Onivyde™** in combination with the 5-FU and oxaliplatin	Preoperative treatment of PC	Phase II	University of Florida
NCT04482257	**Onivyde™** in combination with the 5-FU/LV	Advanced PDAC	Phase I	CSPC Ouyi Pharmaceutical Co., Ltd.
NCT03736720	**Onivyde™** in combination with the 5-FU/LV	Refractory advanced high-grade neuroendocrine Ca of Gastrointestinal, Unknown, or Pancreatic Origin	Phase II	Roswell Park Cancer Institute
NCT04233866	Comparing two treatment combinations, Gemcitabine and **Abraxane™** with 5-Fluorouracil, Leucovorin, and **Onivyde™**	Treatment naïve metastatic PDAC	Phase II	ECOG-ACRIN Cancer Research Group
NCT05074589	**Onivyde™** in combination with 5-FU/LV versus 5-FU/LV in second-line therapy for gemcitabine-refractory pancreatic cancer	Locally advanced or metastatic PC after treatment failure with gemcitabine-based therapy	Phase III	Jiangsu HengRui Medicine Co., Ltd.
NCT03337087	**Onivyde™** in combination with 5-FU/LV and Rucaparib	Metastatic PDAC, CRC, gastroesophageal, or biliary Ca	Phase I/II	Academic and Community Cancer Research United
NCT05047991	**Onivyde™**-containing regimens versus Abraxane® plus gemcitabine	Previously untreated, metastatic PDAC	Phase II	CSPC Ouyi Pharmaceutical Co., Ltd.
NCT04083235	**Onivyde™** in combination with the FOLFOX versus Abraxane®+gemcitabine treatment (NAPOLI 3)	Patients not previously treated for metastatic PDAC	Phase III	Ipsen
NCT03528785	**Onivyde™** in combination with FOLFOX	Resectable PDAC	Phase II	Centro Ricerche Cliniche di Verona
NCT04371224	NaliCap (**Onivyde™**/Capecitabine) versus NAPOLI (**Onivyde™**/5-FU/LV)	Advanced PDAC	Phase II	Seoul National University Hospital
NCT05095064	**Onivyde™** in combination with 5-FU/LV	Metastatic PDAC	A retrospective study on the efficacy and tolerability of Onivyde®	University Hospital, Antwerp
NCT04617457	**Onivyde™** in combination with oxaliplatin, 5-fluorouracil, folinic acid (NAPOX)	Hepatic oligometastatic PDAC	Phase II	University of Cologne
NCT04662112	**Onivyde™** in combination with S-1 (tegafur/gimeracil/ oteracil) and oxaliplatin (NASOX)	Advanced PDAC	Phase I/II	Asan Medical Center
NCT03986294	**Onivyde™** in combination with S-1 versus Onivyde® in combination with 5-FU/LV	Metastatic PDAC	Phase II	Academisch Medisch Centrum - Universiteit van Amsterdam (AMC-UvA)
NCT05363007	Spleen irradiation added to chemotherapy (**Onivyde™** in combination with 5-FU/LV)	Metastatic PDAC	Phase II	National Taiwan University Hospital
NCT05383352	Comparison of **Onivyde™** manufactured at two different production sites administered in combination with 5-FU/LV	Metastatic PDAC	Phase I	Ipsen
NCT03468335	2nd-line therapy with **Onivyde™** after Gem/**Abraxane™**	Locally advanced PDAC, metastatic PDAC	Phase III	AIO-Studien-gGmbH
NCT05251038	Sotorasib in combination with **Onivyde™** and 5-FU/LV versus Sotorasib in combination with gemcitabine and **Abraxane™**	Second-line treatment of PC	Phase I/II	Devalingam Mahalingam
NCT03693677	Gemcitabine in combination with Abraxane® versus **Onivyde™** in combination with 5-FU/LV versus **Onivyde™**/5FU/LV 2-months sequential regimen followed by gemcitabine/ **Abraxane™**	Metastatic PDAC	Phase II	Federation Francophone de Cancerologie Digestive
NCT02826486	BL-8040 (motixafortide, an inhibitor of CXCR4) in combination with pembrolizumab (Keytruda®) versus BL8040/ Pembrolizumab in combination with **Onivyde™** or 5-FU/LV	Metastatic PDAC	Phase II	BioLineRx, Ltd
NCT04825288	**XB2001** (anti-IL-1α True Human antibody) in combination with **Onivyde™** + 5-FU/LV	Advanced PDAC	Phase I/II	XBiotech, Inc.
NCT05277766	**Onivyde™** administered with repeated pressurized intraperitoneal aerosol chemotherapy (PIPAC)	Peritoneal carcinomatosis, peritoneal metastases, CRC, small bowel Ca, appendix Ca, gastric Ca, PC, bile duct Ca	Phase I	University Hospital, Ghent
NCT03703063	Gemcitabine-**Abraxane™** alternating with **Onivyde™**/5-FU/LV (NAPOLI)	Resectable and borderline resectable PDAC	Phase I	Benaroya Research Institute
NCT03487016	Gemcitabine/ **Abraxane™** versus **Onivyde™**/5-FU/LV (NAPOLI) versus seq-NAPOLI-FOLFOX	Metastatic PDAC	Phase II	Ludwig-Maximilians - University of Munich
NCT04258072	Vactosertib in combination with **Onivyde™**/5-FU/LV	Metastatic PDAC	Phase I	Samsung Medical Center
NCT04752696	Onvansertib in combination with **Onivyde™**plus 5-FU/LV	Metastatic PDAC	Phase II	Cardiff Oncology
NCT05472259	**Onivyde™**/5-FU/LV versus **Onivyde™**/5-FU/LV plus oxaliplatin	Metastatic PDAC	Phase II	Belgian Group of Digestive Oncology

(*continued on next page*)

Table 3 (*continued*)

Identifier	Combined therapy	Indication	Phase	Sponsor
NCT03693677	**Onivyde™**/5-FU/LV + **Abraxane™**/Gemcitabine alternatively versus **Onivyde™**/5-FU/LV versus **Abraxanne™**/Gemcitabine	Metastatic PDAC	Phase II	Federation Francophone de Cancerologie Digestive
NCT04247165	Nivolumab and ipilimumab were administered in combination with gemcitabine and **Abraxane™**, followed by immune-chemoradiation.	Borderline resectable, locally advanced or metastatic PDAC	Phase I/II	Herlev Hospital
NCT04257448	Azacitidine and/or Romidepsin® in combination with **Abraxane™**/Gemcitabine	Advanced PDAC	Phase I/II	GWT-TUD GmbH
NCT03193190	Immunotherapy-based treatment combinations: **Abraxane™,** Gemcitabine, Oxaliplatin Leucovorin, Fluorouracil, Atezolizumab, Cobimetinib, **PEGPH20**, BL-8040, Selicrelumab, Bevacizumab, RO6874281, AB928, Tiragolumab, Tocilizumab, LSTA1	PDAC	Phase I/II	Hoffmann-La Roche

Abbreviations: Ca, cancer; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; BC, breast cancer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PEGPH20, PEGylated Recombinant human hyaluronidase enzyme

Table 4
Ongoing clinical trials involving nanocarriers for the treatment of pancreatic cancer.

Identifier	Product name	Indication	Phase	Sponsor
NCT02340117	**SGT-53**: a complex of cationic liposomes encapsulating a human wild-type p53 cDNA in a plasmid backbone. The liposomes surface is decorated with an anti-transferrin (Tf) receptor single-chain antibody fragment (TfRscFv) targeting moiety	Metastatic PDAC	Phase II	SynerGene Therapeutics, Inc.
NCT01591356	**siRNA-EphA2-DOPC**: Small interfering RNA targeting Ephrin type-receptor 2 tyrosine kinase (EphA2) delivered via neutral liposome (1,2-dioleoyl-sn-glycerol-3-phosphatidylcholine or DOPC)	Advanced malignant solid neoplasm	Phase I	MD. Anderson Cancer Center
NCT04640480	**SNB-101**: a lipophilic prodrug of SN38 (the active metabolite of irinotecan), encapsulated into a long-circulating liposomal carrier	Metastatic CRC, PC, BC, gastric, lung, head, and neck Ca	Phase I	SN BioScience
NCT03537690	**FID-007**: paclitaxel encapsulated in a polyethylozaxoline (PEOX) polymer, excipient designed to enhance PK, biodistribution, and tolerability.	Advanced solid tumors that have spread to other places in the body and do not respond to treatment	Phase I	University of Southern California
NCT03382340	**Imx-110:** a micelle encapsulating a Stat3/NF-kB/poly-tyrosine kinase inhibitor (curcumin) and low-dose doxorubicin	Advanced solid tumors, PC, BC, and ovarian Ca	Phase I/II	Immix Biopharma Australia Pty Ltd
NCT04601285	**JS108:** Humanized anti-Trop2 IgG-Tub196 (Tubulysin B analog) conjugate	Advanced solid tumors	Phase I	Shanghai Junshi Bioscience Co., Ltd.
NCT03608631	**iExosomes:** Mesenchymal Stromal Cell-derived Exosomes with KRAS G12D siRNA	Metastatic PDAC with KrasG12D (Kirsten rat sarcoma viral oncogene) mutation	Phase I	MD. Anderson Cancer Center
NCT04175847	**RC-88:** ADC composed of an antibody directed against the human cell surface glycoprotein mesothelin and conjugated, via a cleavable linker, to the microtubule-disrupting cytotoxic agent monomethyl auristatin E (MMAE)	Cancer solid tumors, including mesothelioma, bile duct carcinoma, PC, lung adenocarcinoma, ovarian Ca	Phase I/II	RemeGen Co., Ltd.
NCT03859752	**MT-8633**: Humanized anti-c-Met (hepatic growth factor receptor, HGFR) monoclonal antibody conjugated to a cleavable pyrrolobenzodiazepine toxin	Tumors expressing c-Met, including colorectal, NSCLC, gastric, esophageal, PC, and bile duct Ca	Phase I	Tanabe Research Laboratories USA Inc
NCT04853017	**ELI-002 2 P**: an immunotherapeutic comprised of a lymph-node targeted amphiphile (AMP)-modified G12D and G12R mutant KRAS peptides together with an AMP-modified CpG oligonucleotide adjuvant.	PDAC, CRC, NSCLC, ovarian, biliary, and gallbladder Ca	Phase I	Elicio Therapeutics
NCT04105335	**MTL-CEBPA**, liposomal NPs encapsulating a small activating RNA upregulating C/EBP-α (transcription factor) in combination with Pembrolizumab (PD-1 inhibitor)	Breast, lung, ovarian, pancreatic, gall bladder, HCC, neuroendocrine, and cholangiocarcinoma	Phase I	Mina Alpha Limited
NCT04852367	**ThermoDox®** (Heat–activated liposome-encapsulated doxorubicin) + Focused Ultrasound versus Doxorubicin	Non-resectable PDAC	Phase I	University of Oxford
NCT04161755	Atezolizumab in combination with **Cevumeran** (autogene vaccines RO7198457, uridine mRNA–lipoplex NPs) and FOLFIRINOX	PDAC treatable with surgery	Phase I	Memorial Sloan Kettering Cancer Center

Abbreviations: Ca, cancer; PK – pharmacokinetics, PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; BC, breast cancer; CRC, colorectal cancer; NSCLC, non-small cell lung cancer

nucleic acids that interfere with the expression of specific genes (NCT02340117, NCT01591356, NCT03608631, NCT04105335).

4.2. Nano-based drug delivery systems: new perspectives

Despite significant progress in the design and engineering of NDDSs, the number of approved NP-based drugs for clinical use, compared to those tested in preclinical studies, is limited. A promising strategy to improve the success of new nanomedicine drugs in clinical practice is to move from a "formulation-driven research" to a "disease-driven design" approach [81]. In other words, until now, biomedical research has primarily focused on improving the physicochemical properties of nanocarriers to increase their biological safety, pharmacokinetics, and stability and enhance targeted delivery. However, improving the efficiency of NDDSs will also require us to consider tumor biology, which has received little attention so far.

4.2.1. Smart transformable NDDSs for deep tumor penetration

The increased permeability and retention effect (EPR effect) is the major underlying mechanism enabling the accumulation of NDDSs in the interstitial space of solid tumors relative to healthy tissue (passive targeting). Decorating the NDDS surface with various ligands (aptamers, peptides, or antibodies) can further promote the specific binding of nanocarriers to cancer cells increasing the drug concentration in the

tumor mass (active targeting) [82]. However, the characteristic features of PDAC, including a dense stroma, abnormal vasculature, and increased interstitial fluid pressure (IFP), severely limit efficient nano-based drug delivery into malignant tissue.

Various approaches have been proposed to overcome these obstacles. For example, great efforts have been dedicated to developing multifunctional size-reducible NDDSs for advanced drug delivery [83]. NDDSs are designed so that after reaching the TME, specific endogenous stimuli cause their transformation (e.g., change in size, shape, surface charge) and drug release [84]. Alternatively, NDDS transformation can be triggered by external stimuli such as light, ultrasound, X-ray, magnetic field, or temperature [85].

The size is an important parameter that significantly influences the entrapment and retention of NPs in the tumor tissue. In general, large NPs (NPs $>$ 100 nm) accumulate more easily in the tumor due to vascular extravasation. However, their diffusion into the depth of the tumor is unsatisfactory compared to small NPs ($<$ 20 nm). On the contrary, the disadvantage of small NPs is their fast clearance and, therefore, limited accumulation in the tumor [83]. Wong et al. [86] designed and developed size-changing NDDSs to facilitate drug delivery into the dense collagen matrix of a tumor. In this multistage system, 10 nm NPs were conjugated to the surface of 100 nm gelatin NPs. The size transformation was triggered by MMP-2 overexpressed in the TME, which degrades the cores of gelatin NPs, releasing smaller NPs from the surface. This proof-of-concept was successfully validated in vitro and in vivo. A similar approach was used by Li et al. [87], who developed smart pH-responsive NDDSs with ultrasensitive size switching, providing fast NP diffusion and more efficient tumor penetration. This NDDS was produced from an amphiphilic block copolymer, poly(ethylene glycol)-*b*-poly(2-azepane ethyl methacrylate) (PEG-b-PAEMA) and polyamidoamine (PAMAM) dendrimers encapsulating a platinum-prodrug (Pt). The nanocarrier superstructures self-assemble into pH-sensitive cluster nanobombs (SCNs/Pt) at neutral pH (size ~80 nm), while at the tumor, acidic pH SCNs/Pt dissociate instantaneously into the dendrimer building blocks (size $<$ 10 nm). SCNs/Pt revealed potential anticancer activities in PDAC BxPC-3 cell line spheroids and BxPC-3 tumor xenografts. As GEM monotherapy is considered the gold standard for treating advanced PDAC, a great effort has been devoted to improving the therapeutic efficacy of this deoxycytidine nucleoside analog by developing a suitable GEM nanoformulation. Kulkarni et al. [88] encapsulated GEM in an MMP-9-responsive nanovesicle, which was incorporated into a PEGylated liposome. The PEG groups defended this lipopeptide from premature hydrolysis by MMP-9. The increased intracellular glutathione levels caused the reductive removal of the outer PEG groups, thus exposing MMP-9-responsive lipopeptides to enzymatic cleavage, causing vesicle destruction and drug release. The authors confirmed effective drug release in vitro in two PDAC cell lines and pancreatic tumor xenografts. Several other strategies have been employed to increase the efficacy of GEM for PDAC therapy. Singh et al. [89] fabricated redox-responsive epidermal growth factor receptor-targeted gelatin NPs for systemic administration of GEM. Efficient delivery of GEM was confirmed in orthotopic PDAC tumor-bearing SCID mice. Chen et al. [90] designed and synthesized an aptamer-decorated hypoxia-responsive NDDS. They loaded GEM and the STAT3 inhibitor HJC0152 into small ($<$ 10 nm) dendri-graft poly--lysine (DGL) NPs. The aptamer GBI-10 binds to the ECM after reaching the tumor tissue and spontaneously detaches from the NDDS surface, exposing the positively charged inner core. Hypoxic conditions in the TME caused NDDS disintegration, and size reduction, allowing for deep tumor penetration of DGL NPs. Simultaneously, STAT3 inhibition by HJC0152 softened the tumor stroma and reeducated the TME into an immune-activated state. Gurka et al. [91] synthesized a mesoporous silica nanocarrier (MSN) that was coated with chitosan to allow pH-responsive retention and release of GEM. To increase the efficiency of GEM delivery, urokinase plasminogen activator (UPA) was conjugated to the MSN surface. UPA binds specifically to the UPA receptor overexpressed on the surface of PDAC and stroma cells. Multispectral optoacoustic tomography confirmed the preferential accumulation of these pH-responsive NDDSs in orthotopic pancreatic tumor xenografts. Targeted pH-stimuli responsive micelles for the co-delivery of GEM and PTX, based on a polyethylene glycol−polyarginine−polylysine platform, were designed by Chen et al. [92]. PTX was linked to micelles through a pH-sensitive molecule (2-propionic-3-methylmaleic anhydride, CDM), while GEM was loaded by electrostatic interaction. Targeted delivery was mediated by the AE105 peptide that specifically binds to the UPA receptor (uPAR). After reaching the TME, the acidic pH of the tumor triggered micelle disintegration, as was demonstrated in PDAC tumor xenografts. Wang et al. [93] designed thermally-response NDDSs to increase the efficacy of GEM in PDAC. The photothermally controlled drug release nanosystem (VPNS) consisted of a luminescent core, palladium (PdPc), a photothermal agent, and phosphorylated GEM. After VPNS irradiation with near-infrared light, the photothermal effect from PdPc triggered GEM release. Simultaneously, VPNS enabled photothermal cancer treatment. The antitumor effect of VPNS was demonstrated in vitro in Mia PaCa-2 cells and in vivo in PDAC xenografts. Confeld et al. [94] created a copolymer consisting of PEG and polylactic acid (PLA) NPs (polymersomes) for the targeted delivery of GEM and a STAT3 inhibitor (Napabucasin) to PDAC tumors. A hypoxia-responsive diazo benzene linker incorporated into the polymersomes facilitated cleavage in the hypoxic TME and subsequent drug release. In addition, the small circular tumor-penetrating peptide (iRGD), with specific binding to αVβ3 and αVβ5 integrins, mediated targeted delivery. Cleavage of iRGD by proteolytic enzymes altered its specificity towards the neuropilin-1 receptor, which is overexpressed on pancreatic cancer stem cells (CSCs). The high cellular internalization of the polymersomes in vitro and the increased drug-mediated cytotoxicity in hypoxia confirmed the functionality of these NDDSs. Moreover, the in vivo experiments revealed a significant reduction in tumor size in PDAC xenografts. An interesting approach to increase the efficacy of GEM was proposed by Aspe et al. [95]. The authors showed that exosomes containing the dominant-negative mutant Survivin-T34A could block wild-type Survivin, an inhibitor of apoptosis, thus inducing caspase activation and apoptotic cell death. Such exosomes/Survivin-T34A combined with GEM increased apoptotic cell death in various PDAC cell lines in vitro. This study indicated a novel therapeutic strategy for improving the efficacy of chemotherapy. Li et al. [96] developed a redox-sensitive nanoplatform (PSPGP) for targeted codelivery of the nuclear receptor siRNA (siTR3) and PTX. Endogenous nuclear transcription factor TR3, overexpressed in PDAC, facilitates cell survival and represses apoptosis. Knockdown of the TR3 signaling pathway decreased the expression of antiapoptotic proteins, including Bcl-2 and Survivin. PSPGP was composed of G2 dendrimer-modified 8-armed PEG, and its surface was modified with PTP (plectin-1 targeted peptide), a novel biomarker of PDAC, linked via redox-responsive disulfide bonds. *In vivo*, PSPGP/PTX/siTR3 significantly inhibited tumor growth.

An exciting and promising approach for drug delivery is the materials-based targeting nanoplatform proposed by Colby et al. [97]. This pH-responsive NDDS represents expansile nanoparticles (eNPs) comprised of three basic blocks, one of which obtains a pH-triggered swelling functionality. Under physiological conditions, the mean size of eNC ranges from 30 nm to 50 nm, and the drug is securely packaged within the nanocarrier. However, acidic conditions cause eNPs swelling and drug release. The PTX-loaded eNPs (PTX-eNPs) showed comparable therapeutic efficacy with Taxol in PDAC tumor xenografts, but the toxicity of PTX-eNPs was significantly lower.

4.2.2. Stroma remodeling approaches for efficient drug delivery

The dense fibrous stroma characterized by ECM deposition, extensive fibrosis, vascular collapse, and high tumor interstitial fluid pressure in the TME promotes the poor responsiveness of PDAC to therapy by imposing an almost impermeable physical barrier hindering efficient drug delivery (and immune effector cell infiltration) [98]. Therefore,

targeting and degrading the acellular stromal components to allow deeper penetration of NDDSs into tumor tissue is considered a promising therapeutic strategy for PDAC [99]. Hyaluronic acid (HA) binds to cell surface receptors and activates downstream signaling pathways involved in cell survival, proliferation, migration, and invasion. Moreover, its capacity to absorb and retain water enhances interstitial fluid pressure. Based on this fact, HA is considered an attractive target in PDAC therapy. Jacobetz et al. [100] showed that clinically formulated PEGylated human recombinant PH20 hyaluronidase (PEGPH20) enzymatically depleted HA resulting in increased intratumoral delivery of two therapeutic substances, doxorubicin (DOX) and GEM. PEGPH20 has recently been used in a clinical trial (NCT03193190) combined with other chemotherapeutics. A smart gemcitabine@nanogel (GEM@NGH) system was designed and fabricated by Chen et al. [101]. The GEM@NGH platform consists of a reduction-sensitive core (polyethyleneimine, PEI, cross-linked by disulfide bonds), GEM, and hyaluronidase (HAase), conjugated on the PEI cationic surface. After reaching the TME, HAase cleaves HA, accelerating ECM degradation, and the nanogel is disrupted due to exposure of the disulfide bonds to increased glutathione levels, causing GEM release. The GEM@NGH system showed excellent ECM eradication and exhibited remarkable solid tumor penetration and tumor growth inhibition in PDAC tumor-bearing mice.

Collagen, a major component of the TME ECM, creates a dense matrix network supporting tumor cell survival and promoting tumor progression and metastasis. Zinger et al. [102] constructed a "collagosome", a 100 nm liposome, encapsulating collagenase. Pretreatment of mice bearing allogenic pancreatic tumor xenografts with collagosome, followed by treatment with PTX micelles, induced a significant tumor reduction compared to pretreatment with empty liposome in combination with PTX micelles. Moreover, ECM degradation did not increase the number of circulating tumor cells, which is often a concern when the ECM is targeted [103]. A promising approach for treating desmoplastic malignancies was recently published by Yu et al. [104]. Specifically, they constructed two polymeric methoxy PEG-b-poly(caprolactone) (mPEG-PCL) nanocarriers based on halofuginone (HF) (HKS NPs) and PTX (PKS NPs). HF, a natural, low molecular weight alkaloid, is a potent collagen inhibitor. The in vivo experiments in pancreatic tumor xenograft mouse models confirmed the potent antifibrotic capacity of the HKS NPs, and HKS NP pretreatment considerably facilitated PKS NP penetration into the tumor, causing significant regression of tumor growth. In addition, HF helped to increase the infiltration of cytotoxic T cells. Another strategy to reduce ECM density was described by Wang et al. [105], who constructed the size-tunable SN38 (the active metabolite of irinotecan) prodrug-based polymeric nanocarrier with a hydrophobic inner core, where GDC-0449 (vismodegib), the commercial SHH pathway inhibitor, was encapsulated. To better simulate the TME of pancreatic tumors, co-cultures of immortalized human PSCs and BxPC-3 or MIA PaCa-2 cells were established in vitro and in vivo. In these models, GDC-0449 suppressed the co-culture-induced up-regulation of glioma-associated protein 1 (GLI-1), which triggers the synthesis of collagen and HA, and glucuronosyltransferase (UGT1A), regulating the metabolic inactivation of SN38. This “smart” GDC-0449 and SN38 NDDS co-delivery showed efficient antitumor capacity validated by a reduced desmoplastic stroma, increased intratumoral concentration of SN38, and enhanced sensitivity of tumor cells to SN38.

A size-switchable nanoplatform based on PEG–PLGA nanospheres encapsulated within liposomes for the combined delivery of Vactosertib (VAC), a TGF-β1 receptor kinase inhibitor, and PTX (TAX) was constructed by Zhao et al. [106]. The surface of the liposome was modified with a peptide, APT_{EDB}, (fibronectin extra domain B targeting peptide), allowing anchorage of the nanocarrier to the abundant tumor-associated fibronectin found in the ECM, resulting in decreased size by releasing encapsulated TAX-loaded nanospheres and VAC. In addition, ECM hyperplasia inhibition by VAC facilitated TAX penetration deep into the tumor mass, reducing tumor size, as confirmed in the PDAC xenograft tumor model.

Besides cancer cells, various non-tumor cells represent an integral part of the TME, such as CAFs, TAMs, vascular endothelial cells, pericytes, and immune cells [107]. CAFs represent the greatest proportion of non-tumor stromal cells. Feng et al. [108] developed a CAF-targeted biodegradable polymeric (CRE-NP(α-M)) NDDS to modulate the TME by inhibiting the TGF-β/Smad signaling pathway. They encapsulated α-mangostin (α-M), a natural phytochemical with exceptional chemoprotective and anticancer properties [109], into PEG-PLA polymeric NPs coated with CREKA peptide (generated by phage, with specific affinity to fibronectin overexpressed in CAFs) to achieve active targeting. CRE-NP(α-M) nanocarriers efficiently remodeled the TME, promoted vascular normalization, and enhanced blood perfusion in a PDAC xenograft mouse model. Moreover, they entrapped Triptolide (Trip), a diterpenoid epoxide effective against various malignancies, in a pH-responsive micelle coated with the tumor-penetrating peptide (CRPPR) to target the cancer cell neuropilin-1 (NRP-1) receptor. Treating the tumor with CRE-NP(α-M) followed by CRP-MC(Trip) or GEM efficiently inhibited tumor growth in an orthotopic PDAC tumor model. CRP-MC(Trip) was less toxic and more efficient than GEM. Nanoplatforms developed by Zhao et al. [110] aimed to inhibit SHH signaling using a fabricated polymeric micelle-based nanocarrier (M-CPA/PTX) to co-deliver cyclopamine (CYC), an SHH inhibitor, along with PTX. The performance was tested in a genetically-engineered KPC model of PDAC, and the M-CPA/PTX nanoplatform efficiently modulated the tumor stroma without ablating the collagenous matrix. Inhibition of SHH signaling, leading to CAF inactivation and destruction of stroma deposition, was opted for by Jiang et al. [111]. Specifically, this group developed erythrocyte membrane-camouflaged PLGA NPs for CYC delivery. Such biomimetic PLGA NPs possess favorable biocompatibility and prolonged circulation time. CYC-loaded membrane-coated NPs (CMNPs) effectively delivered their payload to the tumor site, caused stroma ablation, and increased tumor vascularity. Furthermore, CMNP in combination with PTX-loaded MNPs (PMNPs), substantially improved PTX delivery to the TME, resulting in noticeable inhibition in a KPC tumor model. Biomimetic nanocarriers, a red blood cell (RBC) vesicle "shell," was also used by Zhao et al. [112]. They used the RBC camouflage “shell” to partially protect their FnBPA5 peptide, which has a high affinity for CAFs, collagen, and fibronectin. Ion-pair complex DOX • RA (retinoic acid) was encapsulated into the FnBPA5-modified PEG-PLGA nanoplatform (RA, a Golgi-disturbing agent), which was used to down-regulate the secretion of proteins such as α-SMA, FAP-α, collagen I, and fibronectin produced by CAFs. The RBC-Fn-NP platform inhibited tumor growth in allogenic PDAC mouse models. A multifunctional dual-responsive lipid-albumin nanoplatform (HSA-PTX@-CAP-ITSL) was recently proposed by Yu et al. [113]. These NDDSs allowed to combine chemotherapy with photothermal therapy. FAP-α-mediated CAF targeting was achieved by co-assembling a FAP-α responsive cleavable peptide (CAP) with a phospholipid (CAP-TSL). FAP-α is a membrane-bound serine protease expressed explicitly on the surface of CAFs. In addition, IR-780 iodide, a near-infrared (NIR) light-absorbing agent, was incorporated into CAP-TSL to produce CAP-ITSL. Finally, an albumin-PTX (HSA-PTX) conjugate was encapsulated into the CAP-ITSL. Mechanistically, MMP triggered HSA-PTX release in the tumor tissue via the cleavage of FAP-α-responsive CAP. Subsequently, strong hyperthermia caused by NIR laser irradiation killed tumor cells and further enhanced HSA-PTX release and deep tumor penetration. The outstanding antitumor efficacy of HSA-PTX@CAP-ITSL was demonstrated in allogenic PDAC xenograft tumor models.

Mardhian et al. [114] designed and synthesized superparamagnetic iron oxide nanoparticles (SPION) with chemically-conjugated human relaxin-2 (RLX), an endogenous hormone that inhibits CAF activation. They showed that RLX-SPION administration inhibited tumor growth through ECM collagen reduction and desmin (PSC marker) and CD31 (endothelial marker) expression. Dwivedi et al. [115] have also used iron oxide NPs to fabricate DOX-loaded magneto-liposome

microbubbles (DOX-ML-MBs). DOX-ML-MBs were magnetically targeted to tumor tissue, and DOX was released from the carrier upon exposure to ultrasound (US) pulses. A significant reduction in the volume of PDAC xenografts was observed in vivo. Similarly, magnetic iron oxide NPs (MNPs) were used by Han et al. [116] for a sequential two-step delivery strategy for PDAC therapeutic targeting with GEM. First, metformin (MET), a down regulator of fibrogenic cytokine TGF-β expression, was administered to suppress the activity of PSCs and disrupt the dense stroma by inhibiting α-smooth muscle actin and collagen generation. Consequently, GEM and a pH (low) insertion peptide (pHLIP) co-modified MNP (GEM-MNP-pHLIP nanocarrier) were tested. GEM was conjugated to MNPs via the GFLG linker, which is cleavable by cathepsin B overexpressed in PDAC cells. The pHLIP is known to acquire transmembrane capability in the acidic TME by forming a stable transmembrane α-helix that facilitates NP internalization. The sequential MET and GEM-MNP-pHLIP treatment reduced tumor growth in subcutaneous and orthotopic PDAC tumor mouse models.

Hossen et al. [117] demonstrated an exceptional biological capacity of gold NPs (GNPs) to reprogram activated CAFs into quiescent cells by enhancing endogenous lipid synthesis, leading to the accumulation of lipid droplets inside cells. Such unique properties of GNPs could be utilized in a wide range of applications where remodeling activated fibroblasts can help to improve pathological outcomes.

A promising alternative to enhance drug delivery to the desmoplastic stroma was proposed by Wei et al. [118]. They prepared DOX-loaded thermosensitive liposomes (MC-T-DOX). The nanocarrier's surface was modified with low-density cilengitide, an $\alpha v\beta 3$ integrin-specific Arg-Gly-Asp (RGD)-cyclic mimetic peptide, conjugated to an MMP-stimuli responsive peptide. Integrin-mediated promotion of endothelial cell migration could improve tumor hypoperfusion, thus increasing drug delivery to the tumor mass. After cleavage by MMP, the locally released cilengitide increased tumor blood perfusion, thus improving MC-T-DOX retention in the tumor tissue. In addition, DOX released from the liposome by temperature stimulus inhibited tumor growth in subcutaneous PDAC xenograft models.

CSCs are a small subpopulation of highly tumorigenic pluripotent cells within a tumor, with unlimited self-renewal and inherent chemoresistant and metastatic capacities [119]. From a treatment perspective, it is believed that eliminating CSCs could have clinical benefits. Therefore, targeting CSCs has been a strategy for PDAC intervention for some time. Verma et al. [120] prepared α-mangostin-encapsulated PLGA NPs (Mang-NPs), and their functionality was tested in human and KC mouse-derived (Pdx^{Cre};LSL-$Kras^{G12D}$) pancreatic CSCs in vitro and in KPC mice in vivo. Mang-NPs inhibited cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) and suppressed CSC characteristics in pancreatic CSCs in vitro. In KPC mice, Mang-NPs inhibited tumor growth and hindered liver metastasis. In addition, the molecular analysis of tumor tissue revealed suppression of the SHH-Gli pathway and stemness markers ($CD24^+$, $CD133^+$), modulation of pluripotency-maintaining factors, EMT markers expression, and upregulation of E-cadherin. Their results suggested that Mang-NPs could be a promising tool for treating PDAC by targeting CSCs. Similarly, the superparamagnetic iron oxide NP formulation containing curcumin (SP-CUR), developed by Khan et al. [121], targeted the SHH pathway and the CXCR4/CXCL12 signaling axis. Moreover, the SP-CUR nanocarriers enhanced GEM efficacy in orthotopic PDAC tumor models. Tumor tissue analysis revealed downregulation of the expression of pluripotency-maintaining factors, indicating that SP-CUR nanocarriers targeted PDAC cells, including the CSC sub-population.

Exosomes have emerged as promising candidates for drug delivery in preclinical and clinical settings due to their numerous advantages over traditional drug delivery systems. Their main benefit is their immunological inertness and lack of cytotoxicity. Likewise, they can be engineered to carry therapeutic cargo (chemotherapeutic drugs, miRNAs) [122], and their surfaces can be modified with specific ligands or antibodies, providing tissue-specific delivery and minimizing off-target effects. In addition, exosomes carry different transmembrane and membrane-anchored proteins extending their half-life in blood circulation and their ability to cross multiple biological barriers. Their potential as drug delivery systems for PDAC has been reviewed recently by Xu et al. [123]. One of the most successful approaches was demonstrated by Kamerkar et al. [124], who engineered exosomes derived from normal fibroblast-like mesenchymal cells to carry siRNAs and shRNAs targeting the $KRAS^{G12D}$ oncogene (iExosomes). The authors showed that iExosomes were more efficient in suppressing tumor growth in allogenic and xenogeneic PDAC mouse models than iLiposomes loaded with RNAi targeting oncogenic KRAS, despite a comparable loading efficacy in both nanocarriers due to the expression of CD47 (an integrin) protecting the exosomes from phagocytosis. A Phase I trial (NCT03608631) is currently underway to investigate the efficacy of these iExosomes in treating stage IV PDAC. Promising results were also obtained by Shang et al. [125] with bone marrow mesenchymal stem cells (BM-MSCs) transfected with miR-1231 oligonucleotides (miR-1231 mimics). Administration of miR-1231 mimic BM-MSC exosomes, significantly inhibited tumor growth in PDAC tumor xenograft models.

Despite encouraging results following the re-modulation of the PDAC stroma in preclinical models, several studies have indicated that stromal depletion could promote tumor cell proliferation, invasion, and metastasis [126–128]. Therefore, Fan et al. [129] proposed a different approach to eradicate the dense tumor stroma. They prepared a micelle composed of cellular membrane-disruptive molecules (synthetic mimetics of host defense peptides) to eliminate both stromal and cancer cells within the primary tumor. These acid-activatable micelles dissociate into component molecules at weakly acidic pH, an inherent characteristic of the PDAC TME, disrupting the integrity of cellular membranes of stromal and cancer cells. However, these micelles are intact at physiological pH, allowing for long-lasting circulation with no cytotoxicity in normal tissues. Indeed, these NPs effectively permeabilized the stromal barrier and inhibited PDAC xenograft tumor growth in in vivo studies.

Combining chemotherapy and cell-penetrating peptides can be a promising approach to overcome therapy resistance. For example, He et al. [130] designed and fabricated a sequentially triggered nanoplatform (Apt/CPP-CPTD NPs) consisting of redox-responsive dimeric camptothecin (CPTD) prodrug-based NPs, a cell-penetrating peptide (CPP), and GBI-10, a ssDNA aptamer with a high affinity and specificity for tenascin-C, an ECM glycoprotein. After GBI-10 detachment, due to binding to tenascin-C, CPP deeply penetrated the ECM. The intracellular redox potential triggered the controlled gradual release of the CPTD prodrug, resulting in increased drug accumulation in tumors in vivo and good treatment efficacy. Table 5 summarizes the examples of smart NDDSs designed to target non-cellular and cellular TME constituents.

4.2.3. Modulation of the tumor immunosuppressive microenvironment

While PDAC is an inherently immunologically "cold" tumor, and at present, immunotherapy has not proven effective, several alternative and innovative immunotherapy-based strategies have been designed/proposed to target this tumor [131]. Among them, targeting growth factors and growth factor receptors could be a promising approach for modifying the immunosuppressive TME. For example, Miao et al. [132] designed a plasmid DNA encoding a fusion (antibody-like) protein with high binding affinity to CXCL12 (called CXCL12 trap). CXCL12 secreted by CAFs causes the suppression of immune surveillance, thus helping tumor cells avoid immune system detection [133,134]. Locally administered lipid-protamine-DNA (LPD)-encapsulated CXCL12 traps blocked CXCL12 signaling and potentiated $CD8^+$ T-cell penetration into the tumor mass. The therapeutic efficacy was further increased by co-delivering the CXCL12 trap with the LPD-loaded plasmid encoding the programmed cell death ligand-1 (PD-L1) trap. The PD-L1 trap permitted infiltrated T-cells to destroy the PDAC cells, leading to significant tumor shrinkage. This phenomenon was accompanied by reduced systemic toxicity in the combination trap therapy, as observed

Table 5
NDDSs targeting TME in pancreatic cancer.

Target	Therapeutic approach	Nanocarrier	Ligand/Stimuli	Drug/s	Ref.
Non-cellular stroma components					
Hyaluronic acid	Monotherapy	PEGPH20		Human recombinant PH20 hyaluronidase	[100]
	Monotherapy	GEM@NGH (nanogel)	Disulfide bonds	Hyaluronidase/ gemcitabine	[101]
Collagen	Monotherapy	Collagosome (liposome)		collagenase	[102]
	Combined therapy	HKS NPs PKS NPs (polymeric nanocarrier)		Halofuginone/ PTX	[104]
HA + collagen	Co-delivery	Polymeric nanocarrier		SN38/ GDC-0449	[105]
Fibronectin	Co-delivery	PEG-PLGA nanospheres + liposome	APT_{EDB} peptide	Vactosertib/ PTX	[106]
Cellular stroma components					
CAFs	Combined therapy	CRE-NP(α-M) CRP-MC(Trip) micelle	CREKA peptide pH-stimuli/ tumor-penetrating peptide (CRPPR)	α-mangostin (α-M) Triptolide (Trip)	[108]
	Co-delivery	M-CPA/PTX NPs		CYC/ PTX	[110]
	Combined therapy	CMNPs Polymeric NPs		CYC	[111]
		PMNPs Polymeric NPs		PTX	
	Co-delivery	RBC-Fn-NP platform	FnBPA5 peptide	DOX/RA	[112]
	Combined therapy with photothermal therapy	HSA-PTX@CAP-ITSL Lipid-albumin nanocarrier	CAP-TSL	HSA-PTX/ IR-780 iodide	[113]
	Monotherapy	RLX-SPION		RLX	[114]
	Combined therapy	DOX-ML-MBs	Magnetic field/ultrasound		[115]
	Combined therapy	Pretreatment with MET, followed by GEM-MNP-pHLIP nanocarrier	pHLIP/cathepsin B	GEM	[116]
	Monotherapy	Gold NPs			[117]
Endothelial cells	Combined therapy	MC-T-DOX (liposomes)	Cilengitide/MMP stimuli/ temperature stimuli	DOX	[118]
CSCs	Monotherapy	Mang-NPs polymeric NPs		α-Mangostin	[120]
	Combined therapy	SP-CUR in combination with GEM		Curcumin	[121]
	monotherapy	iExosomes	CD47	siRNAs and shRNAs targeting the $KRAS^{G12D}$ oncogene	[124]
	Monotherapy	BM-MSCs derived exosomes		miR-1231 mimics	[125]
Stromal+cancer cells		micelle composed of cellular membrane-disruptive molecules - synthetic mimetics of host defense peptides	pH stimuli		
		Apt/CPP-CPTD NPs	Redox-stimuli/cell penetrating peptide/ GBI-10 aptamer	Camptothecin	[130]

in a subcutaneous PDX model of PDAC. Similarly, Shen et al. [135] designed and constructed LPD-loaded DNA plasmids encoding trap proteins targeting both interleukin 10 (IL-10), an immunosuppressive cytokine, and CXCL12. Using orthotopic allograft PDAC models, the authors confirmed the higher efficacy of combined trap gene therapy compared to trap treatment alone. Moreover, in addition to inhibiting tumor growth, a significantly reduced number of immunosuppressive cells was also observed in the TME. An impressive triple combination ($CXCR4/miR\text{-}210/siKRAS^{G12D}$) therapy to improve antitumor immunity was proposed by Xie et al. [136] in which the authors used simple cholesterol-modified polymeric CXCR4 antagonist (PCX) NPs for the codelivery of anti-miR-210 and $siKRAS^{G12D}$. The CXCR4/CXCL12 axis is involved in cancer immune evasion, miR-210 overexpression helps cancer cells adapt to hypoxic conditions, and *KRAS* mutations play a crucial role in tumor initiation, progression, and survival. Blocking of CXCL12 binding to its receptor CXCR4, interrupted the interaction between cancer cells and the stroma. Moreover, miR-210 and $KRAS^{G12D}$ silencing caused the inhibition of the stroma-producing PSCs and the efficient killing of PDAC cells, respectively. *In vivo* experiments in orthotopic KPC-derived PDAC tumor models revealed that the triple-action combination therapy platform had superior therapeutic effects compared to the individual treatments. Specifically, significant tumor growth inhibition, desmoplastic stroma depletion, and enhanced cytotoxic T-cell infiltration into the TME were observed in treated animals.

Combination therapy based on co-inhibition of the TGF-β pathway and the PD-L1 checkpoint has been proposed by Wang et al. [137]. pH-responsive clustered NPs (iCluster) consisting of self-assembly poly (ethylene glycol)-*b*-poly(ε-caprolactone) (PEG-PCL), PCL homopolymer, and poly(amidoamine)-*graft*-polycaprolactone (PCL-CDM-PAMAM) were used to encapsulate LY2157299, a TGF-β receptor inhibitor, in the carrier's core, while siPD-L1 was electrostatically adsorbed on the surface by positively charged PAMAM. In the TME, the acidic conditions triggered siPD-L1 and PAMAM release, facilitating its penetration deeper into the stroma with the corresponding blockade of the PD-1/PD-L1 immune checkpoint. At the same time, LY2157299 inhibited the activation of PSCs, leading to decreased collagen production and a significant increase in infiltrated $CD8^+$ T cells. Simultaneously, $CD8^+$ T cell infiltration significantly increased. This combination therapy approach significantly suppressed the growth of orthotopic PDAC tumors in vivo.

Re-education of TAMs, the most abundant immune cell population in the TME [138,139], has also attracted attention as a perspective strategy for treating PDAC [140,141]. TAMs are characterized by their immunosuppressive properties and pro-tumorigenic M2-like phenotypes; thus, a promising approach to target these immune cells lies in repolarizing tumor stromal TAMs into tumor-inhibiting pro-inflammatory M1-like macrophages by RNA interference (RNAi), as proposed Cao et al. [142]. Cao and colleagues synthesized a GSH-responsive nanoplatform consisting of a solid polydisulfide amide (PDSA)/cationic lipid

core and a lipid-poly(ethylene glycol) (lipid-PEG) shell, in which monoacylglycerol lipase (MGLL) and endocannabinoid receptor-2 (CB-2) siRNAs were encapsulated. Silencing the MGLL, overexpressed in tumor cells and associated with cancer progression, cut off the nutrient supply to PDAC tumor cells. On the other hand, inhibition of CB-2 overexpressed on TAMs repolarized them towards a tumor-inhibiting M1-like state, thus reversing the immunosuppressive TME. The antitumor potential of the (siMGLL/siCB-2) platform was validated in allogenic PDAC tumor models. Another strategy used by Parayath et al. [143] for reprogramming M2 TAMs towards an M1-like phenotype involved encapsulating miR-125 (that regulates macrophage activation) into M2 peptide (YEQDPWGVKWWY)-hyaluronic acid-polyethyleneimine (HA-PEI/PEG) conjugates. The M2 peptide, which preferential binds to M2 macrophages, allowed specific targeting of TAMs, while the HA polymer targeted the CD44 receptor on the surface of macrophages, reprograming TAMs from a pro-tumoral to anti-tumoral state in PDAC tumor models. Another example that enhances immunotherapy and TME reprogramming involves the exosome-based bioplatform designed by Zhou et al. [144] that enhances immunotherapy and TME reprogramming. Bone marrow mesenchymal stem cell exosomes were loaded with a galectin-9 siRNA and oxaliplatin (OXA) prodrug. Galectin-9, which is highly expressed in PDAC, can ligate dectin 1, a key immune receptor expressed on the surface of macrophages, producing pro-tumoral M2-like macrophages. OXA can trigger immunogenic cell death, leading to dendritic cell (DC) maturation and promoting infiltration of cytotoxic T lymphocytes in the TME. The proposed biosystem showed a synergistic immune response mediated by inducing immunogenic cell death (ICD) stimuli and reversing immunosuppression and DC activation, resulting in a remarkable reduction of tumor size in orthotopic allogenic PDAC tumor models. Blockage of phosphatidylinositol 3-kinase γ (PI3K-γ) and colony-stimulating factor-1 (CSF-1)/CSF-1 receptor pathways used by Li et al. [145] have also been proposed to achieve remodeling of TAMs and the immunosuppressive TME in PDAC. PI3K-γ and CSF-1/CSF-1R pathways are involved in the infiltration and alternative polarization of M2 TAMs [146]. The authors developed an M2 TAM targeting micelle to co-deliver the PI3K-γ inhibitor (NVP-BEZ 235) and a CSF-1R-siRNA. This micelle suppressed PDAC tumor growth, converted M2 TAMs into M1 TAMs, enhanced $CD8^+$ and $CD4^+$ T cell infiltration, and inhibited the infiltration of MDSCs. The underlying strategy of immunostimulatory NPs (immuno-NP) proposed by Lorkowski et al. [147] was to reprogram and activate local antigen-presenting cells (APCs) in the TME into T cell-stimulatory cells. The immuno-NPs, consisting of DPPC (1,2-dipalmitoyl-sn-glycerol-3-phosphocholine), cholesterol, and $mPEG_{2000}$-DSPE ((methoxy-poly (ethylene glycol)− 2000 1,2-distearoyl-sn-glycero-3--phosphoethanolamine-N), were used to co-deliver two immune agonists, both of which induced interferon beta (IFN-β). Cyclic diguanylate monophosphate (cdGMP) is an agonist of the stimulator of interferon genes (STING) pathway, while monophosphoryl lipid A (MPLA) is a Toll-like receptor 4 (TLR4) agonist. Immuno-NPs were shown to accumulate in the perivascular regions of the tumor and were efficiently taken up by DCs, leading to APC expansion and lymphocyte infiltration. These promising results indicated that such an approach could enhance innate immunity in hard-to-reach and highly immunosuppressive PDAC tumors.

Metabolic reprogramming also contributes to the regulation of macrophage activation (reviewed in Lui et al. [148]). Overexpression of indoleamine 2,3-dioxygenase 1 (IDO1) in the TME, a tryptophan catabolic enzyme, causes immunosuppression that can be reversed by a small molecule inhibitor, indoximod (IND). Lu et al. [149] developed a dual delivery carrier for OXA and IND using lipid-bilayer coated mesoporous silica nanoparticles (OX/IND-MSNP). OXA, the ICD-inducing drug, was encapsulated in the porous MSNP interior, while IND was conjugated to a phospholipid and incorporated into a lipid bilayer. Experiments with orthotopic PDAC tumor models revealed a synergistic immune response (disappearance of Tregs and enhanced innate immunity) induced by the OXA/IND-MSNP platform, which was concomitant with significant tumor regression. Moreover, MSNPs improved drug delivery into the tumor mass. For reversing IDO1-mediated immunosuppression, Huang et al. [150] encapsulated an IDO1 siRNA into cationic lipid-assisted nanoparticles (CLANs). OXA and $CLAN_{siIDO1}$ combination therapy generated synergetic antitumor effects, resulting in DC maturation, increased tumor-infiltrating T lymphocytes, and decreased Treg cells in allogenic PDAC tumor models.

Promising results were reported by Sun et al. [151] using extracellular vesicles (EVs) derived from natural killer (NK) cells that were co-cultured with pancreatic cancer cells. EVs secreted by NK cells were enriched in miR-3607–3p, which inhibited malignant transformation and PC development in vitro and in vivo. The suppression of migration and invasion of tumor cells was mediated via direct targeting of IL-26 by miR-3607–3p. Crosstalk between immune and cancer cells could be a promising strategy for PDAC treatment. Jang et al. [152] proposed an interesting approach combining photodynamic and immune therapy. They constructed tumor-derived reassembled exosomes loaded with a chlorin e6 photosensitizer (Ce6-R-Exo). Ce6-R-Exo allowed visualization by photoacoustic imaging and efficiently generated reactive oxygen species due to laser irradiation. Moreover, Ce6-R-Exo stimulated the release of cytokines from immune cells owing to the presence of the tumor antigen located in the cell membrane of the tumor-derived exosomes.

Cancer vaccines represent a very promising strategy to overcome PDAC immunosuppression. Several mucins, which are aberrantly overexpressed in PDAC and have been shown to promote tumor growth [153], have emerged as ideal tumor-associated antigens (TAAs). Banerjee et al. [154] proposed a MUC4β-based polyanhydride nanovaccine as an effective immunotherapeutic modality for PDAC. They encapsulated recombinant human mucin (MUC4β) in NPs, composed of 1,8-bis (p-carboxyphenoxy)− 3,6-dioxaoctane (CPTEG) and 1,6-bis(p-carboxyphenoxy) hexane (CPH). The nanocarrier stabilized the structure and activity of the encapsulated protein, and a surface erosion mechanism mediated its release. Data from an in vivo study revealed that immunization with the MUC4β-nanovaccine solicited a strong anti-MUC4 humoral response and increased circulating levels of inflammatory cytokines. Another strategy for developing cancer vaccines was proposed by Affandi et al. [155]. This group formulated a liposome decorated with gangliosides, natural receptors to CD169, to target human CD169-expressing APCs with encapsulated tumor antigen. Uptake of these nanovaccine carriers, co-delivering tumor antigen and Toll-like receptor ligand, by $CD169^+$ DCs, led to cytokine production, cross-presentation, and the activation of tumor-specific $CD8^+$ T cells. *Ex vivo* experiments showed effective ganglioside-liposome capture by $CD169^+$ DCs isolated from cancer patients. The results indicated that these liposomes targeting human $CD169^+$ DCs could be a unique vaccination platform to stimulate antitumor T cell responses. Table 6 summarizes the examples of smart NDDSs designed to target the immunosuppressive TME in pancreatic cancer.

4.2.4. Nano-based delivery of epigenetic drugs

NDDSs also offer a promising approach for enhancing epigenetic drug effectiveness in managing solid tumors, including pancreatic cancer [156]. The NDDSs can shield epigenetic drugs from degradation and elimination by the immune system, thus extending their circulation in peripheral blood and enhancing their therapeutic potential. Intensive research has focused on optimizing the physicochemical properties of nanocarriers to enhance the stability of epigenetic drugs and reduce off-target effects. Limited studies have tested the functionality of such NDDSs in PDAC, although various models of other solid tumors, including breast, liver, stomach and lung, have been used.

Despite the potential of DNA demethylating agents such as AZA and DAC for treating solid tumors, their rapid clearance, poor solubility, short half-life, and off-target effects have limited their use. To overcome these shortcomings, Naz et al. [157] chemically conjugated AZA with

Table 6
NDDSs targeting the immunosuppressive TME in pancreatic cancer.

Target		Carrier name	Ligand/ stimuli	Drug/s	Ref.
CAFs signaling	Co-delivery	LPD (lipidic NPs)		plasmid encoding CXCL12 / PD-L1	[133]
	Co-delivery	LPD		plasmid encoding IL-10/ CXCL12	[135]
	Co-delivery	PCX (cholesterol-modified polymeric CXCR4 antagonist		anti-miR-210/ siKRAS^{G12D}	[136]
	Co-delivery	iCluster	pH stimuli	LY2157299/ siPD-L1	[137]
TAMs	Co-delivery	PDSA/ lipid-PEG (siMGLL/siCB-2)	Redox stimuli	siMGLL/ siCB-2	[142]
	monotherapy	HA-PEI/PEG conjugates	HA/M2 peptide	miR-125	[143]
	Co-delivery	BM-MSC derived exosomes		galectin-9 siRNA/ OXA	[144]
	Co-delivery	micelles		NVP-BEZ 235/ CSF-1R-siRNA	[145]
APCs (dendritic cells)	Co-delivery	Immuno-NP (polymeric NPs)		cdGM/ MPLA	[147]
Metabolic reprogramming	Co-delivery	OX/IND-MSNP		IND/ OXA	[149]
	Combined therapy	CLANs		IDO1 siRNA	[150]
Crosstalk between immune and cancer cells	monotherapy	EVs		miR-3607–3p	[151]
TME	Combined therapy (Immuno+photodynamic)	Ce6-R-Exo		chlorin e6	[152]
	Cancer vaccine	CPTEG/ CPH	Surface erosion	MUC4β	[154]
	Cancer vaccine	liposomes		gangliosides	[155]

the di-block backbone of PLGA-PEG, allowing pH-sensitive drug release. This conjugated AZA nano-based form has demonstrated better therapeutic efficacy in mouse xenograft models of breast cancer than free AZA, including enhanced drug solubility and bioavailability, higher concentration in cancer cells, and more robust anti-proliferative activity. Li et al. [158] synthesized polymeric (MPEG-*b*-PLA) NPs to encapsulate DAC (NP_{DAC}) and DOX (NP_{DOX}). Combined exposure of breast cells to NP_{DAC} and NP_{DOX} reduced the number of CSCs in vitro and suppressed tumor growth and DNMT1 and DNMT3b expression in mouse breast xenografts in vivo. Hong et al. [159] encapsulated DAC into gelatinase-stimuli polymeric NPs consisting of PEG and PCL (DAC-TNPs) to sensitize gastric cells to 5-FU. DAC release was triggered by MMP2/9 overexpressed in the TME. Sequential in vitro and in vivo treatment with DAC-TNPs, followed by 5-FU, resulted in improved therapeutic efficacy of 5-FU. An alternative oral mode of DAC delivery was proposed by Neupane et al. [160]. They demonstrated that their lipid-based nanocarrier for potential oral DAC delivery showed a 4-fold increase in gut permeation of DAC compared to free drug.

Diverse nanocarriers have also been designed to improve HDACi delivery [161]. For example, Alp et al. [162] encapsulated CG-1521 (HDACi) into starch NPs, Singleton et al. [163] uploaded panobinostat (LBH589) into pluronic (P407) micelles, Sankar et al. [164] encapsulated SAHA into biodegradable polymeric PLGA, and Wang et al. [165] loaded SAHA and quisinostat into PLGA-lecithin-PEG NPs. A more sophisticated nanocarrier for HDACi delivery was developed by Peng et al. [166]. SAHA was encapsulated into a pH-sensitive PLGA-DOTAP core polymer coated with hybrid membranes derived from RBC and metastatic lung cancer cells (HRPDS). SAHA release was triggered by pH stimuli in the TME. Ruttala et al. [167] constructed a transferrin (Trf)-anchored albumin nanocarrier with PEGylated lipid bilayers (Tf-L-APVN) for the targeted codelivery of PXT and SAHA to solid tumors. PTX and SAHA release from Tf-L-APVN was triggered by acidic pH. pH-stimuli for releasing HDACi were also used by Bertrand et al. [168]. The authors synthesized polymeric NDDSs loaded with vorinostat (SAHA), tacedinaline (CI-994), and trichostatin A (TSA). The norbornenyl-poly(ethylene oxide) NPs were prepared using the innovative Ring-Opening Metathesis Polymerization (ROMP) technique, and the HDACi prodrugs were conjugated to the nanocarrier via a covalent link adapted for release at acidic pH using click chemistry. The prolonged circulation of the NDDSs was mediated by poly(ethylene oxide) (PEO), which shielded the epigenetic drugs from premature release. The authors showed an increase in intratumoral histone H3 acetylation, while no changes in acetylation were observed in other organs in a syngeneic mesothelioma tumor model. However, a significant (80%) reduction of the tumor mass and a decrease in pancreatic invasion were observed only in TSA-loaded NDDS-exposed animals. The authors reasoned that the low loading efficacy of their nanoplatform was the cause for the failure observed with the other epigenetic drugs. Tran et al. [169] developed solid lipid NPs (SLNs) coated with HA for targeted delivery of vorinostat (SAHA) to cancer cells overexpressing CD44. Vorinostat loaded in SLNs showed longer half-time circulation and higher plasma concentration than the free drug. To increase the targeted delivery of SAHA, Zong et al. [170] synthesized linker-modified SAHA to prepare PAMAM dendrimer-SAHA conjugates decorated with folic acid (FA). The authors showed the cell-specific uptake of FA-coated dendrimer-SAHA conjugates only in folate receptor (FR)-expressing cells. The functionality of ester-linker-modified SAHA was confirmed by increased histone acetylation, causing apoptosis using the cancer cell model. Xu et al. [171] developed a cleavable SAHA-based prodrug polymer (POEG-b-PSAHA), which can self-assemble into prodrug micelles and serve as nanocarriers for DOX delivery. The polymer (POEG-b-PSAHA) consists of hydrophilic poly (oligo (ethylene glycol) methacrylate) (POEG) block and hydrophobic SAHA segments. Co-delivery of DOX and SAHA using this polymeric NDDS showed a synergistic effect in vitro and in a mouse breast cancer model representing the solid tumor. Finally, Li et al. [172] encapsulated SAHA into M1 macrophage-derived exosome membrane-modified mesoporous silica upconversion NPs (EMS). The integrin α4β1 on M1-EMS was essential for homing EMS to tumor tissues, which facilitated SAHA accumulation in the tumors and efficient epigenetic inhibition.

Another example includes 4-phenyl butyric acid (PBA), an HDACi conjugated to HA via ester bonds that were developed to amplify curcumin's anticancer potential [173]. The intracellular PBA released from the HA–PBA conjugate was triggered by esterase-responsive cleavage. CD44, the receptor of HA, mediated PBA targeted delivery. Nanodiamonds (NDs) were employed as carriers for the delivery of UNC0646, a G9a inhibitor [174]. G9a or EHMT2 (euchromatic histone-lysine *N*-methyltransferase 2) catalyzes the dimethylation of histone H3 lysine 9 (H3K9). In the context of PDAC, Huang et al. [175] recently presented a promising strategy to overcome tumor drug resistance, developing the J/T @ 8P4 nanoplatform consisting of 8P4, hydrophobic L-phenylalanine-poly(ester amide), JQ1 (BRD4i), and THZ1 (CDK7i). The J/T @ 8P4 nanoplatform allowed controlled drug release and improved drug delivery. The in vitro and in vivo (patient-derived

xenografts) experiments demonstrated that the THZ1/JQ1 nanoformulation is a promising therapeutic strategy for GEM-resistant PDAC. Xiao et al. [176] designed and synthesized stimuli-responsive disulfide cross-linked micelle (DCMs) to encapsulate thailandepsin A (TDP-A), a natural HDACi. The release of TDP-A was triggered by the cleavage of disulfide bonds in the presence of elevated glutathione (GSH) levels in tumor cells. TDP-A/DCMs possessed better water solubility than free TDP-A and showed comparable cytotoxicity in breast cancer cells in vitro. Using an orthotopic breast xenograft model, the authors demonstrated preferential accumulation of TDP-A/DCMs in tumors and enhanced histone H3 acetylation compared to normal organs. Moreover, the combined administration of TDP-A/DCMs and bortezomib (BTZ, Velcade®), a potent proteasome inhibitor encapsulated in DCMs resulted in synergistic anti-tumor effects.

An exciting strategy to enhance the therapeutic effects of epigenetic drugs is to encapsulate a cocktail of DNMTi and HDACi into NDDSs. Vijayaraghavalu et al. [177] demonstrated better efficacy in overcoming drug resistance by pre-treating breast cancer cells with biodegradable nanogels loaded with DAC and SAHA followed by exposure to DOX encapsulated in nanogels compared to exposure to the same free drugs. Kim et al. [178] encapsulated DAC and panobinostat (PAN) into lipid nanoemulsions (LNEs) decorated with lysophosphatidic acid receptor 1 (LPAR1), overexpressed in triple-negative breast cancer tissue, to allow for active targeting. The authors showed that the co-delivery of DAC/-PAN using LPAR1-targeted LNEs restored CDH1/E-cadherin expression and down-regulated FOXM1 expression, causing reduced viability of breast cancer cells. Moreover, LNE biodistribution in vivo was LPAR dependent.

Effective translation of preclinical findings from various solid cancers to PDAC requires a biomarker-driven approach that combines traditional and novel therapies, including next-generation epigenetic drugs.

5. Available PDAC models for testing the efficacy of NDDS: pros and cons

Despite extensive exploration of NDDSs in preclinical studies, their current evaluation in clinical trials for PDAC remains limited, and only a few nano-based drugs have received approval for clinical use [179]. One of the primary obstacles is the absence of suitable and physiologically-relevant preclinical models. Consequently, the availability of reliable models that accurately mimic the complexity of the PDAC TME, characterized by high levels of dense ECM and genetic heterogeneity, becomes crucial.

PDXs in immunodeficient mice and genetically-engineered mouse models (GEMMs) represent the two most extensively used in vivo models for PDAC and serve as valuable tools for studying PDAC biology, conducting preclinical drug testing, and validating NDDSs. PDX mouse models can reflect inter-patient heterogeneity and have demonstrated significant potential in replicating patient responses [180]. It is worth noting that PDXs are derived from the primary resected tumor of PDAC patients, who tend to have a longer OS compared to patients with metastatic disease. Although the establishment can take several months, a feasible time frame exists for their use in personalized medicine. However, PDX models are not always possible to establish for the majority of PDAC patients with advanced metastatic disease. For these patients, there is no tissue from surgical intervention and we rely on tissue samples from a biopsy that is limited due to clinical guidelines. In general, primary and/or metastatic tumor tissue for establishing PDXs is difficult to obtain for this patient population. Another downside of the PDX model is the lack of a functional immune system, which limits studies involving immunotherapy or immune modulation. As an alternative to the aforementioned immunodeficient mouse models, humanized mice bearing mutations in the IL2 receptor common gamma chain (IL2rgnull) in a non-obese diabetic (NOD)/SCID background were developed. These mice support engraftment with human tissue, peripheral blood mononuclear cells, and hematopoietic stem cells, enabling the modeling of human immunity in immunocompromised mice with PDXs [181].

GEMMs of PDAC avoid many of the common problems of xenografts, exhibit pathophysiological characteristics of human PDAC and are useful to assess immunotherapy potential as these models are immunocompetent. The most representative GEMM was developed by Hingorani and coworkers. The KPC model contains a *TP53*^{R172H} mutation along with a *KRAS*^{G}12D mutation, resulting in tumors that faithfully recapitulate most of the pathological features of human PDAC [182]. Moreover, the KPC model closely mimics the complex TME of human PDAC and has been used in biomarker development and testing targeted therapies as well as immunotherapies [183]. However, their main disadvantage is that the mouse genome does not entirely reflect the human genome, the high costs associated with breeding and genotyping, and experiments using these mice can be very time-consuming due to the limited number of mice born with all the transgenes of interest [184]. Thus, in vitro models tend to represent faster and more scalable platforms for establishing clinically beneficial patient-specific models of PDAC. Two-dimensional (2D) monolayer cell lines have been used extensively in the past decades to test various therapeutics; however, many 2D studies failed to translate into clinically-relevant solutions because 2D cultures lack the complexity of the 3D tumor mass. Nowadays, 3D in vitro culture models, such as spheroids, patient-derived organoids, air-liquid interface cultures (ALI), or explant cultures, represent the state-of-the-art for translational studies [185]. They are all complementary methods with several levels of complexity and biological relevance.

Spheroids, solid 3D clusters, are generated from established 2D cell lines. They can be grown in liquid media or embedded in or on an ECM [186]. Organoids are unique 3D culture models that can self-organize in contained structures. Pancreatic 3D organoids can be generated relatively quickly from small amounts of tissue, allowing drug and therapy testing [187]. Benefits of cancer organoids include the retention of cancer cell heterogeneity, histological features, and/or genetic and mutational status. Patient-derived 3D organoid technology has provided a unique opportunity to study patient-specific properties. However, neither spheroids nor organoids can faithfully recapitulate the complex PDAC TME. High CSC content, high density of stromal cells, and ECM are only partially reproduced in spheroid and organoid cultures. Co-culture systems of organoids with PSCs, CAFs, or immune cells can overcome this gap, but such multi-cellular cultures are difficult to establish and maintain.

Organotypic slice cultures, a specific in vitro model using precision-cut slices of tissue (150–350 μm thick) submerged in medium [186], represents the next level of physiological models. Their advantage lies in the maintenance of the TME and the spatial information of the tumor. Organotypic slice cultures have been implemented in the PDAC research field relatively recently. For example, Peña et al. developed an *ex vivo* model of PDAC based on the 3D co-assembly of peptide amphiphiles (PAs) capable of generating nanofibrous hydrogels mimicking the architecture of the natural ECM, with custom ECM components (PA-ECM) [188]. Thus, the PA-ECM model's main advantage is maintaining patient-specific transcriptional profiles while exhibiting CSC functionality and strong in vivo tumorigenicity. Of note, patient-specific in vivo drug responses were better reproduced in PA-ECM cultures than in other models. A whole-tissue *ex vivo* explant model (1–2 mm explants cultured on gelatin sponges for 12 days) that maintains viability, 3D multicellular architecture, and microenvironmental cues of human PDAC tumors, represents another promising approach [184].

Furthermore, organ-on-a-chip systems provide novel platforms for incorporating diverse cell types under flow conditions, mimicking the physiological situation, and including vascularization or ECM components. Organs-on-a-chip are microfluidic systems that enable the generation of a defined microenvironment for growing cells, including PDAC cells [189], with patient-derived organoids combined with

microfluidic platforms representing a future-orientated technique with high potential for personalized medicine approaches [190]. In addition to microfluidic platforms for high-throughput PDAC organoid cultures [191,192], an organotypic PDAC-on-a-chip culture model that emulates vascular invasion and tumor-blood vessel interactions to better understand PDAC-vascular interactions has been described by Nguyen et al. [193]. This tumor-on-a-chip model provides an important in vitro platform to investigate the process of PDAC-driven endothelial ablation and may provide a mechanism for tumor hypovascularity. Furthermore, a ductal tumor micro-environment-on-a-chip applied by Bradney et al. [194] provided further insight into organ-on-a-chip systems' relevance to understand the complexity of intratumoral heterogeneity. Their platform could be used to study tumor cell invasiveness and aggressiveness as well as to develop patient-derived tumor-stroma interaction models. While for pre-screening of drug efficiency, it would be feasible to use these organ-on-a-chip approaches for patient stratification and personalized therapies [195,196], intensive development is still needed to bring such systems into everyday use.

6. Bridging the gap between preclinical efficacy and clinical outcome

The clinical translation of preclinical efficacy remains a formidable challenge in drug development, specifically in the context of nanomedicine [197]. While Phase 1 trials of cancer nanomedicines have shown a high success rate of 94%, the success rate drops to around 48% in Phase 2 trials, as reported by He et al. [198]. Most failures in Phase 2 trials were due to poor efficacy and, to a lesser extent, toxicity. Phase 3 trials exhibit an even lower success rate of about 14%, primarily due to low efficacy. Despite the overall limited success in clinical trials, nanomedicines have a relatively higher approval rate than conventional cancer drugs, with a success rate of 6% vs. 3.4%, respectively, from Phase 1 to approval, as reported by Wong et al. [199]. This demonstrates the potential of nanomedicines in addressing unmet medical needs.

However, the effectiveness of nanomedicines in animal studies does not always translate to expected outcomes in patients. Variations in tissue distribution, pharmacokinetics, target site accumulation, and drug release, especially in the complex TME of PDAC, contribute to this discrepancy [200]. Additionally, the biodistribution of drug molecules can significantly change when delivered via NPs, potentially leading to localized overexposure in specific organs and NP-related toxicity. To address these safety concerns, thorough preclinical pharmacokinetic and biodistribution studies, along with histopathological and clinical chemistry assessments, are essential to evaluate organ-specific drug exposure and toxicity. Furthermore, predicting immunological responses, including hypersensitivity reactions, complement cascade activation, and interactions between NPs and blood cells based on small laboratory animal studies, particularly in PDX models, remains challenging. In vitro complement binding assays, cell interaction studies, and preclinical safety assessments in larger animal models are necessary to address these difficulties [201].

In addition, patient stratification in clinical trials plays a crucial role in determining the success of translating preclinical efficacy into clinical outcomes. For instance, Opaxio™, a biodegradable polymeric polyglutamate-paclitaxel conjugate, has demonstrated significant benefits in women with premenopausal estradiol levels due to its activation by cathepsin B, which correlates with estrogen levels [202]. On the other hand, the failure of BIND-014, a polymeric nanoparticle encapsulating docetaxel [203], CRLX101, a camptothecin-bevacizumab conjugate [204], and NK105, a PTX encapsulated in polymeric micelles [205], can be attributed to inconclusive response rates observed in unstratified patient cohorts. As the clinical translation of preclinical efficacy in nanomedicine is a complex process, understanding the factors influencing efficacy, safety, and immunological responses is crucial for developing and applying nanomedicines in cancer treatment. Therefore, the identification of biomarkers for patient stratification is crucial for refining clinical trials involving nanomedicines for cancer treatment.

7. Measures for safe utilization of NDDS in the clinic and future perspective

The safety assessment of NPs is essential for biomedical applications in order to recognize potential risks and create preventive measures. However, different NP properties, such as their size, surface modifications, and surface charge, make it challenging to categorize risks. Although several recommendations are found in the scientific literature and in guidance documents from regulatory bodies that could be applied to nanomedicine products, including NDDSs [206–210], there is so far no specific legislative or regulatory framework for the regulation of nanomedicines [211]. Nevertheless, a general agreement exists in the international regulatory community that nanotechnology-enabled health products can be regulated according to existing legislative/regulatory frameworks with a case-by-case approach to address nano-specific features of nanomedicines. In addition, regulatory authorities such as EMA, FDA, and the Ministry of Health, Labour and Welfare of Japan (MHLW) are collaborating and seeking the harmonization of regulatory practices in order to support the mutual acceptance of data in different regions and have released specific guidance on data requirements for certain classes of nanotechnology-based products [211]. For this reason, an international platform, the International Pharmaceutical Regulators Program (IPRP), has been established to harmonize drug regulatory activities and guidelines worldwide [212]. The objective of the International Council for the Harmonization of Technical Requirements for the Registration of Medicinal Products for Human Use (ICH) is to put together the regional regulatory authorities and the pharmaceutical industry representatives to debate the scientific and technical aspects of the registration of medicinal products.

The physicochemical characteristics of nanomedicines, including NDDSs, are very complex; therefore, the current regulatory practice may not be sufficient to assess their safety/risk ratio. Robust datasets guiding the regulatory needs for quality, safety, and efficacy evaluation are lacking, and the development of additional guidance, methods, and approaches is needed to address nano-specific features of nanomedicines. New strategies are, therefore, crucial to increase the efficacy of NDDSs, including their potential to reduce the side effects of cancer treatment.

Although it is well accepted that during the last decades, mortality in many types of cancer, including breast and colon, has been reduced due to a deeper understanding of the tumor's biology, improved diagnostic devices, and available treatment strategies, this does not apply to PDAC. Improvement in both treatment options and OS of PDAC patients remains rather limited. In addition to the intra-tumoral heterogeneity, which includes CSCs that are highly metastatic and present variable treatment response, the highly desmoplastic stroma and immunosuppressive TME that are characteristic of PDAC represent the major obstacles hampering treatment efficiency.

A better knowledge of not only a patient's genetic but also epigenetic profile is also considered necessary for refining and tailoring medical care to each individual's needs, leaving behind the "one-size-fits-all" therapeutic approach. Nowadays, paraffin-embedded tissue and/or liquid biopsies have become a part of these precision medicine molecular study approaches.

8. Conclusion

PDAC poses significant challenges in terms of curability, as it typically does not respond well to standard therapies and has a high likelihood of recurrence. However, recent research on epigenetic deregulation in various cancers has revealed the potential of targeting reversible epigenetic changes as a novel therapeutic approach. Despite the experimental nature of this approach in solid tumors, there are notable obstacles such as limited tolerability, low efficacy, and off-target

effects associated with most epigenetic drugs. To address these challenges, alternative strategies such as lower drug doses, sequential scheduling, and targeted delivery have become promising candidates for improving their therapeutic index.

Nanotechnology has played a revolutionary role in enhancing drug delivery for epigenetic therapy in solid tumors. Preclinical and clinical trials investigating nano-based epigenetic drugs for PDAC treatment have demonstrated reduced systemic toxicity and improved efficacy compared to traditional free-drug formulations. Personalized-targeted therapy should leverage all available resources, including nano-based delivery systems, new-generation epigenetic drugs, and robust biomarker data, to improve treatment efficacy for PDAC.

Nanomedicine seems to open new horizons as combining multiple agents within a single multi-component nano-drug can enhance the efficacy of conventional therapy. Still, while this approach is promising, a Safe(r)-by-Design strategy for nano-based delivery systems and a vision that would ensure the prevention of adverse health effects and environmental risks are crucial to be adopted.

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Declaration of Competing Interest

The authors declare no competing interests.

Data Availability

No data was used for the research described in the article.

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