

Review

# An Update on Gemcitabine-Based Chemosensitization Strategies in Pancreatic Ductal Adenocarcinoma

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## Abstract

Pancreatic cancer is the seventh leading cause of cancer-related deaths, and chemotherapy is one of the most important treatments for pancreatic cancer. Unfortunately, pancreatic cancer cells can block chemotherapy drugs from entering the tumor. This is owing to interactions between the tumor's environment and the cancer cells. Here, we review the latest research on the mechanisms by which pancreatic cancer cells block the chemotherapy drug, gemcitabine. The results of our review can help identify potential therapeutic targets for the blocking of gemcitabine by pancreatic cancer cells and may provide new strategies to help chemotherapy drugs penetrate tumors.

**Keywords:** pancreatic cancer; chemotherapy resistance; chemosensitization; tumor microenvironment; gemcitabine

## 1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of pancreatic cancers, which have one of the worst prognoses among solid malignancies in humans. Pancreatic cancer is the seventh leading cause of cancer death, with a 5-year survival rate of only 10% [1–3]. Surgical resection is the only possible cure for early-stage PDAC; however, only 15%–20% of patients are eligible for surgery, and the overall 5-year survival rate after surgery is only 10%–25% [4,5]. Therefore, chemotherapy remains a crucial treatment option for PDAC.

Effective delivery of gemcitabine (GEM) to tumor foci is a critical aspect of PDAC treatment. However, the presence of highly fibrotic and densely vascularized tumor tissues poses challenges to drug delivery, leading to limited delivery and difficulty in achieving adequate drug concentrations [6]. Importantly, the interaction between PDAC cells and the tumor microenvironment (TME) is known to stimulate extensive fibrous proliferation [7]. Patients with PDAC have shown poor response rates to immune checkpoint inhibitors, such as anti-programmed cell death protein 1 (PD-1), anti-programmed death ligand 1 (PD-L1), and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), alone or in combination [8]. However, Bockorny *et al.* [8] recently showed that the combination of CXCR4 and PD-1 may enhance the chemotherapeutic efficacy for PDAC, and this finding was later confirmed in a subsequent randomized controlled trial (RCT). The TME in PDAC is characterized by its complexity and diverse cellular components, including cancer-associated fibroblasts (CAFs),

tumor-associated macrophages (TAMs), and immune cells. These cellular elements can generate chemical signals that are associated with GEM and can, consequently, impact the efficacy of the drug [9].

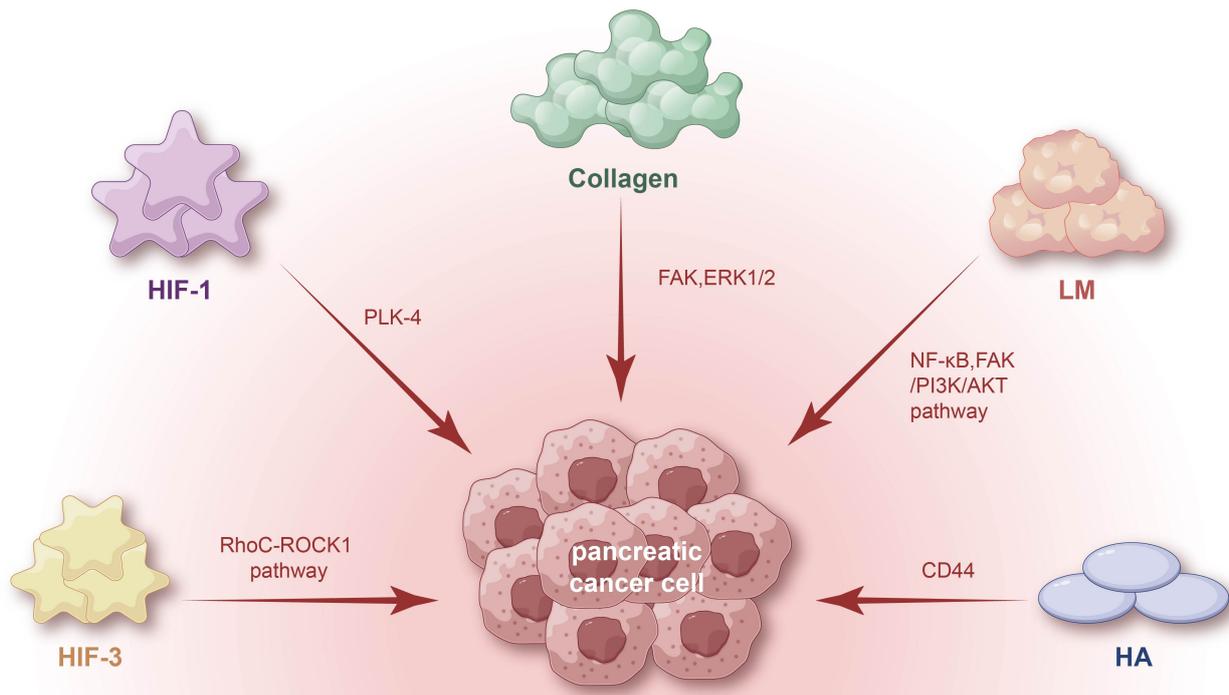
GEM has been the basis of first-line chemotherapy for patients with PDAC. Although FOLFIRINOX has shown some benefits in certain studies, a recent randomized phase II clinical trial (NCT02562716) demonstrated that, compared to the AG regimen, the FOLFIRINOX regimen did not prolong survival, instead it substantially increased drug toxicity [10]. The mechanism underlying GEM cytotoxicity involves its role as a deoxycytidine nucleoside analog that competes for DNA strand incorporation and terminates its replication, ultimately leading to cell death [11]. Our team elucidated the role of metabolic remodeling in GEM-resistant pancreatic cancer chemotherapy [12]. Recently, several key developments have been uncovered regarding the involvement of TME in GEM chemotherapy resistance mechanisms in PDAC; however, few reviews are currently available. Therefore, this review aims to provide the current research progress on the mechanism of GEM chemoresistance, with the goal of identifying potential therapeutic targets for GEM resistance and providing new strategies for chemosensitization.

## 2. Non-Cellular Components of TME in Chemotherapy Resistance

### 2.1 Hypoxia

Extensive fibrosis and reduced blood supply vessels are among the important features of PDAC, which lead to





**Fig. 1. In the tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC), an abundance of fibrous tissue is observed, leading to a persistent hypoxic condition.** This fibrosis is also responsible for impeding drug penetration due to its high levels. HIF, hypoxia-inducible factor; LM, Laminin; RhoC, Ras homolog family member C; ROCK1, Rho-associated coiled-coil containing protein kinase 1; HA, Hyaluronan; FAK, focal adhesion kinase; PLK4, polo-like kinase4; NF- $\kappa$ B, nuclear factor kappa B.

severe tissue hypoxia. Persistent hypoxia has been demonstrated in pancreatic cancer, with a 17-fold decrease in oxygen utilization in PDAC tissues compared with that of other tumors. The average oxygenation level in the healthy pancreas is 6.8%, whereas it is only 0.4% in pancreatic cancer [13]. Hypoxia-induced activation of hypoxia-inducible factor (HIF) signaling is present in almost all types of malignancies [14]. Additionally, *HIF-1 $\alpha$* , *HIF-2 $\alpha$* , and *HIF-3 $\alpha$*  isoforms have been identified. In PDAC, the primary sources of extracellular H<sup>+</sup> are *HIF-1 $\alpha$* -mediated glycolytic lactate production and carbonic anhydrase (CA)-mediated carbonic acid production [15]. *HIF-1 $\alpha$*  induces CA expression by upregulating polo-like kinase 4 (*PLK4*), mediating increased PDAC glycolysis, changes in intracellular and extracellular pH, and increasing GEM resistance [16] (Fig. 1). Similarly, another study demonstrated that *HIF-1 $\alpha$*  is an upstream regulator of retention in endoplasmic reticulum sorting receptor 1 (RER1), and knockdown of RER1 inhibited PDAC cell proliferation, migration, and invasion [17]. Deletion of *HIF-2 $\alpha$*  inhibited tumor progression in PDAC mouse models by reducing macrophage chemotaxis and M2-type polarization, suggesting that *HIF-2 $\alpha$*  is a potential target for chemosensitization [18]. A promising clinical trial of an *HIF-2* inhibitor (belzutifan) II is underway (NCT04924075). Additionally, the expression of *HIF-3 $\alpha$*  is more substantial in PDAC cells than that of *HIF-1 $\alpha$*  and *HIF-2 $\alpha$*  under hypoxic conditions. *HIF-3 $\alpha$*

promotes invasion and metastasis of pancreatic cancer cells *in vivo* by activating the Ras homolog family member C (RhoC)-Rho-associated coiled-coil containing protein kinase 1 (ROCK1) signaling pathway, and targeted inhibition of the *HIF-3 $\alpha$* /RhoC-ROCK1 signaling pathway may be a novel approach to reverse chemoresistance in pancreatic cancer [19] (Fig. 1).

## 2.2 Biological Barriers

Collagen is a major component of the TME and plays a role in forming dense lattices in cancer fibrosis. Type I collagen, which is closely related to PDAC, is a heterotrimer and includes many Gly-X-Y repeat sequences [20]. Type I collagen activates focal adhesion kinase (FAK), a multifunctional non-receptor protein tyrosine kinase, by binding to  $\beta$ 1 integrin and associating with multiple  $\alpha$  subunits [21]. This induces self-renewal and invasion of pancreatic cancer stem cells (CSCs) [22], in addition to fibrous proliferation that enhances the physical barrier to chemotherapeutic drug delivery [23]. Defactinib is a potent oral FAK inhibitor, and a recent phase I clinical trial (NCT02546531) study has shown that the triple drug combination of defactinib, pembrolizumab, and GEM in patients with PDAC prolonged their overall survival [24]. Additionally, collagen restricts the effect of GEM by upregulating membrane type-1 matrix metalloproteinase (MT1-MMP)-dependent ERK1/2 phosphorylation and allowing MT1-MMP-dependent overex-

pression of high mobility group protein A2 (HMGA2) [25]. HMGA2 has three lysine- and arginine-rich AT-hook domains that bind to AT-rich sequences in DNA minor grooves. These AT-rich sequences and AT structural domains promote cytoprotective DNA base excision repair by their cleavage enzyme activity [26], thereby reducing the killing effect of GEM. Therefore, targeting collagen-related pathways may be a new GEM sensitization strategy [27] (Fig. 1).

Laminin (LM) and fibronectin are important basement membrane components involved in blood vessel formation [28]. LM-332, composed of  $\alpha 3$ ,  $\beta 3$ , and  $\gamma 2$  subunits, is a major member of the LM family, which is overexpressed in PDAC. Targeted inhibition of the three subunits hinders tumor cell proliferation, migration, and invasion; thus, the three subunits of LM-332 are expected to be new chemotherapeutic sensitization targets [29]. A xenograft model showed that fibroblasts stimulated by transglutaminase 2 (TG2) secreted by PDAC cells prompted the secretion of LM-A1, which protected cancer cells from GEM-induced cytotoxicity. This process is associated with the activation of nuclear factor kappa B (NF- $\kappa$ B) and FAK/PI3K/AKT pro-survival signaling pathways [30,31]. Additionally, LM promotes survival protein expression (survivin) by inducing FAK/Akt phosphorylation, which resists GEM-induced cytotoxicity and apoptosis. Therefore, selective inhibition of FAK phosphorylation may be another approach to enhance the chemosensitivity of pancreatic cancer [32] (Fig. 1).

Fibronectin is less abundant in the extracellular matrix (ECM), but can interact with various proteins for signal transduction, promote angiogenesis [33] and play an essential role in cell adhesion and mobility [34]. Fibronectin can resist GEM-induced apoptosis by inducing ERK1/2 phosphorylation, thereby mediating GEM drug resistance [35]. However, fibronectin was abundantly expressed in PDAC but did not affect patient survival [36]. Therefore, fibronectin may play a dual role in the development of PDAC.

Hyaluronan (HA) is a proteoglycan secreted by stromal cells highly expressed in the PDAC stroma. It has been associated with elevated interstitial fluid pressure, compression of intra-tumor vessels, decrease in tumor perfusion and impaired vascular permeability, thus, leading to increased physical barriers for chemotherapeutic drug delivery and resulting in GEM resistance [37]. In addition, HA should bind specific receptors to function, primarily CD44, a non-kinase transmembrane receptor with multiple isoforms located on the cell membrane. GEM can induce CD44 subtype switching and promote switching to the more resistant and aggressive PDAC phenotype [38]. Activation of epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF1R) can induce CD44 subtype switching and promote chemoresistance. However, activation of these growth factor receptors may not be required to

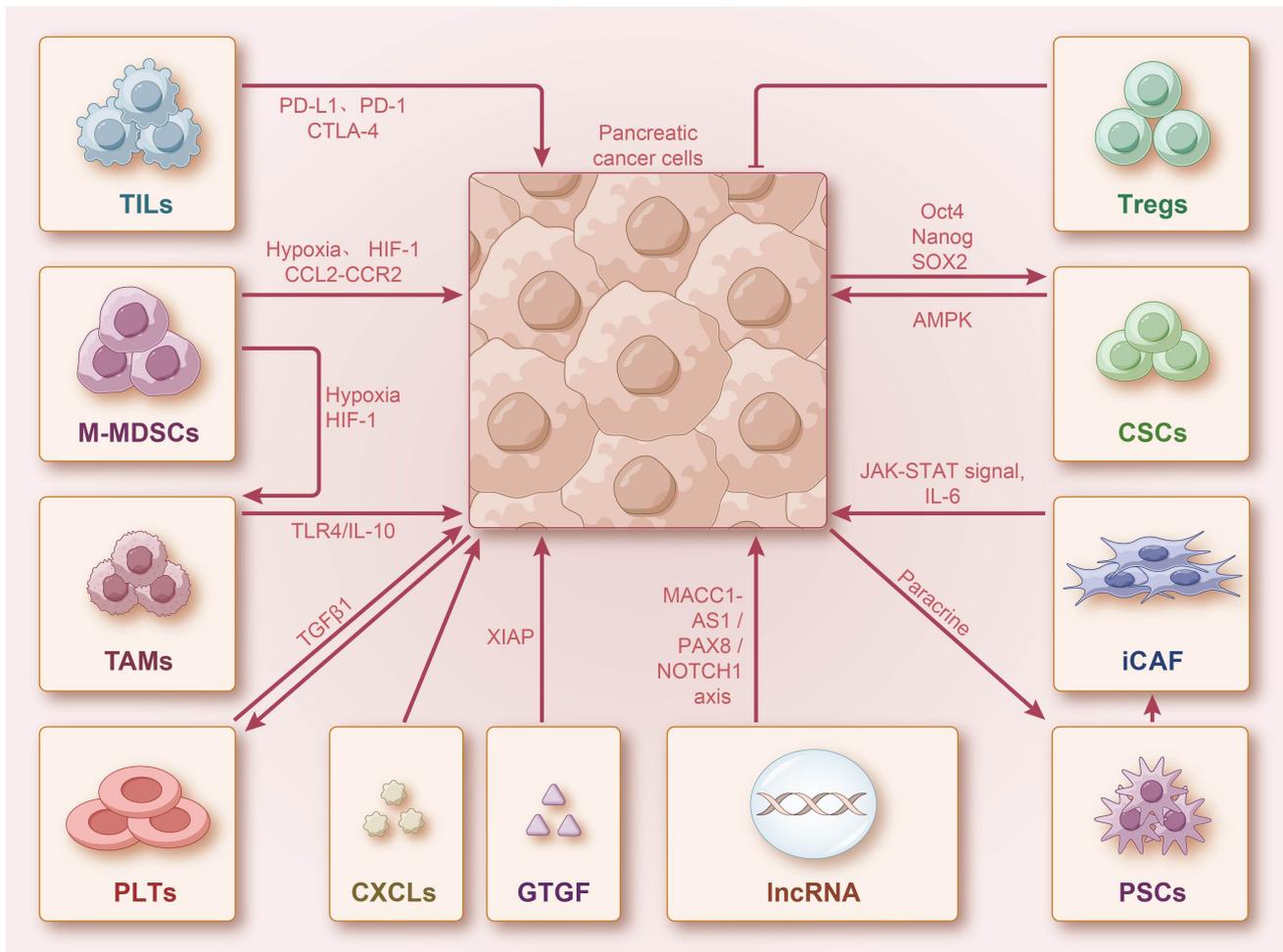
maintain the resistant phenotype, and blocking IGF1R signaling has been shown to contribute to downregulation of GEM-induced CD44 isoform conversion, thereby inhibiting cell invasiveness and improving chemoresistance [39] (Fig. 1). Moreover, 4-methylumbelliferone (4-MU), an inhibitor of HA synthesis, has been shown to reduce HA production and PDAC cell migration. Therefore, 4-MU could be used to improve GEM sensitivity [40].

### 3. Cellular Components of the TME in Chemotherapy Resistance

The cellular components of the TME in chemoresistance include CAFs, TAMs, immune cells, and various other types of cells. These diverse cell types play a crucial role in mediating chemoresistance. Moreover, the TME also comprises additional cell types, such as mesenchymal cells and mesenchymal stem cells that have the potential to considerably influence tumor development and chemotherapy resistance.

#### 3.1 Mesenchymal Cells

Activated pancreatic stellate cells (aPSCs) can interact with PDAC cells directly and through the paracrine (ECM protein) pathway, which is additionally involved in GEM resistance [41–44]. In PDAC, CAFs are mainly derived from the activation of PSCs [41,45,46] and are induced to form two subtypes, inflammatory (iCAF) and myofibroblast (myCAF), by the direct action of PDAC cells and paracrine signaling [47]. myCAFs are naturally resistant to GEM and can induce acquired resistance through multiple mechanisms [45,48]. iCAFs promote tumor chemoresistance through a series of cytokines and chemokines [49]. Mechanistically, iCAFs further activate the JAK-STAT signaling pathway through interleukin-1 (IL-1) stimulation to induce chemoresistance. Preclinical studies have shown that JAK inhibitors can reverse this effect [50] (Fig. 2). Unfortunately, ruxolitinib (a JAK1 and JAK2 inhibitor) did not improve the overall survival of patients with locally advanced or metastatic PDAC in two phase III RCTs (NCT02117479 and NCT02119663) [51]. Furthermore, genomic analysis has revealed that IL-6 is overexpressed in GEM-resistant iCAFs and may be a key inhibitor of immune checkpoint blockade therapy in PDAC [52]. A phase II trial (NCT02767557) is currently underway, which targets IL-6 inhibitors in combination with GEM, nab-paclitaxel, and golimumab [53]. Notably, GEM treatment has been found to induce CAFs to secrete an increased number of exosomes. CAFs present in tumors release exosomes that exhibit high expression of miR-3173-5p. This particular microRNA promotes drug tolerance by suppressing ferroptosis through the miR-3173-5p/ACSL4 pathway [54]. Ferroptosis is a newly identified form of iron-dependent programmed cell death that has been associated with GEM chemoresistance in PDAC [55]. ACSL4 plays a crucial role in regulating ferroptosis by activating



**Fig. 2. PDAC cells interact with stromal cells. Stromal cells can interact with PDAC cells through direct contact or paracrine secretion, enhancing the efficacy of gemcitabine (GEM).** Additionally, stromal and immune cells can secrete various cytokines and chemokines, activating multiple signaling pathways to interact with PDAC. They can also induce GEM resistance by promoting stem cell self-renewal. Long non-coding RNA (lncRNA) can also affect PDAC cell proliferation and induce GEM resistance through multiple signaling pathways. PD-1, programmed death-1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T lymphocyte associated antigen-4; HIF, hypoxia-inducible factor; CSCs, cancer stem cells; PSCs, pancreatic stellate cells; CAFs, cancer-associated fibroblasts; IL, interleukin; lncRNA, long non-coding RNA; TAMs, tumor-associated macrophages; MDSCs, myeloid-derived suppressor cells; CXCL, chemokine ligands; TILs, tumor-infiltrating lymphocytes; PLTs, platelets; M-MDSCs, monocytic-myeloid-derived suppressor cells; GTGF, connective tissue growth factor; Tregs, regulatory T cells; TGF $\beta$ 1, transforming growth factor  $\beta$ 1.

fatty acid metabolism. Numerous exosome studies are ongoing [56], which may provide new insights into overcoming GEM resistance.

### 3.2 Tumor Stem Cells

CSCs account for approximately 0.5%–1% of pancreatic cancer cells. Furthermore, CSCs can self-renew; hence, playing a key role in the development of PDAC and chemoresistance [57,58]. Preclinical studies have shown that methyl-CpG binding domain 3 (MBD3) is overexpressed in PDAC. The knockdown of MBD3 reduces *Oct4*, *Nanog*, and *Sox2* expression; impairs the self-renewal capacity of CSCs; and inhibits the proliferation of PDAC cells [59] (Fig. 2). Similarly, inhibition of *Oct4* and *Nanog* ex-

pression suppressed the tumorigenic phenotype of CSCs and increased the chemosensitivity of CSCs in mice [60]. Betulinic acid (BA) can downregulate the expression of *Sox2*, *Oct4*, and *Nanog* by activating the AMPK signaling pathway [61]. Therefore, BA is expected to be a potential GEM chemotherapy-sensitizing agent. Moreover, *NR5A2* overexpressed in PDAC cells plays an important role in maintaining stemness and promoting epithelial-mesenchymal transition (EMT) in CSCs [62], suggesting that it could be a potential therapeutic target. There is growing evidence that the dysregulation of long non-coding RNAs (lncRNAs) is closely associated with the development and metastasis of PDAC. HOX transcript antisense RNA is a class of lncRNAs, which can promote prolifera-

tion and hinder apoptosis of CSCs by inhibiting miR-34a-activated JAK2/STAT3 and inducing GEM resistance [63, 64]. MACC1-AS1 was recently identified using lncRNA microarray as the most abundant lncRNA in PDAC—it promotes PDAC cell proliferation and metastasis through MACC1-AS1/PAX8/NOTCH1 axis [65,66]. The current research on CSCs and related non-coding RNAs has received extensive attention, and it is predicted that they are highly likely to constitute another strategy for GEM sensitization.

### 3.3 Immune Cells

TAMs consist of two main subtypes, i.e., M1 and M2, with the M1 type activating the immune response against the tumor and increasing chemotherapy sensitivity, contrary to the M2 type [67]. Therefore, induction of TAM reprogramming to M1 or specific inhibition of M2 conversion can help increase GEM sensitivity. CCL2/CCR2 axis activation can recruit inflammatory monocytes to the TME and achieve M2-type conversion, and CCR2 inhibitors can effectively block TAM recruitment [68]. A phase Ib clinical trial (NCT01413022) confirmed that a CCR inhibitor (PF-04136309) in combination with GEM enhances chemotherapeutic efficacy, and further RCT results are expected [69]. M2 can also promote EMT in PDAC through TLR4/IL-10 signaling and induce GEM resistance [70]. Therefore, the TLR4/IL-10 pathway may also be a promising target for chemosensitization.

Myeloid-derived suppressor cells (MDSCs) are heterogeneous immune cell populations of immature myeloid cells that suppress T-cell immunity and promote angiogenesis [71]. Among them, monocyte-like MDSCs (M-MDSCs) have a high plasticity in the TME [72]. The TME can increase the number of M-MDSCs via secretion of CCL2, CCL5, and CSF1 [73]. In addition, under hypoxic conditions with upregulated *HIF-1 $\alpha$* , M-MDSCs can differentiate into TAMs, thus, promoting PDAC progression and GEM resistance [74] (Fig. 2). Inhibition of the CCL2–CCR2 signaling pathway considerably reduces MDSCs migration, decreases TAM numbers, and inhibits tumor growth [75]. Hypoxia inhibits the STAT3 pathway by upregulating CD45 tyrosine phosphatase activity in MDSCs and induces differentiation of M-MDSCs to TAM; therefore, this pathway may also be a potential target for chemotherapy sensitization [76].

Circulating neutrophils can also be recruited by tumors via chemokine action; however, the role of TME neutrophils in chemotherapy may be bidirectional, which may be related to distinct differentiation subtypes [77]. Inhibition of the CXCR2 axis blocks neutrophil recruitment and increases T-cell recruitment [78]. *In vitro* experiments have confirmed that CXCR2 inhibitors can enhance antitumor immunity and toxic effects of GEM [77,79]. Notably, neutrophil extracellular trap (NET) is a newly discovered form of neutrophil activation that has been shown to play

a crucial role in innate immunity and tumor progression [80,81]. NET can promote EMT in PDAC cells through the IL-1 $\beta$ /EGFR/ERK pathway and is considered to be a new potential target for chemosensitization [82].

Tumor-infiltrating lymphocytes (TILs) are key cells involved in antitumor immunity and regulation of immune checkpoints. The results of several studies on PD-1/PD-L1/CTLA-4 inhibitors suggest that GEM combined with immunotherapy is a viable option, although many questions remain regarding the underlying mechanisms [83] (Fig. 2). GEM combined with anti-PD-1 antibodies can enhance the immune response of Th1 lymphocytes, resulting in benefits for patients with advanced PDAC [84]. Based on histopathological features, TME immune cells can be classified into three subtypes: immune enriched, escape, and exhaustion. Among them, the immune escape subtype is the most common and characterized by a low content of T and B cells and an enrichment of regulatory T cells (Tregs) [85]. However, Treg depletion failed to alleviate immunosuppression in PDAC tumors, but instead led to accelerated tumor progression. Thus, Tregs infiltration may have a protective role in PDAC; although the exact mechanism is unknown [86,87]. In summary, studies on the complex mechanisms underlying the role of TILs in TME immunity are emerging and may provide new insights for the comprehensive treatment of PDAC.

The role of platelets (PLTs) in tumor development remains controversial [88]. Recently, it has been shown that PDAC cells can induce the recruitment activation of PLTs. This further promotes EMT and induces GEM resistance through direct contact or release of transforming growth factor  $\beta$ 1 [89–91]. Therefore, PLTs are prospective chemotherapy sensitization targets.

## 4. Interaction between Cellular and Non-Cellular Components of TME in Chemotherapy Resistance

Cytokines and chemokines play a key role in mediating the interaction between PDAC cells and ECM, and in the induction of GEM resistance (Fig. 2).

### 4.1 Cytokines

Connective tissue growth factor (CTGF) can interact with other growth factors to mediate chemoresistance. CTGF and FGF2 have been shown to jointly induce ERK phosphorylation through fibroblast growth factor receptor 2 (FGFR2) signaling, leading to resistance to GEM-induced cytotoxicity and promoting chemoresistance [92]. Moreover, TGF- $\beta$  promotes CTGF production in PC cell lines and further induces FGFR2 expression. Sensitization to GEM chemotherapy can be achieved with a CTGF antagonist (mAbFG-3019) [93]. A subsequent phase II study (NCT02210559) showed that pamrevlumab (FG-3019) enhanced the sensitivity to GEM in patients with locally advanced pancreatic cancer [94].

**Table 1. Potential targets and mechanisms of multiple cell and cytokine induction of gemcitabine (GEM) resistance in pancreatic ductal adenocarcinoma (PDAC) cells in the tumor microenvironment (TME).**

Potential targets	Drug resistance mechanism	Drug/premedication	Clinical trials	Phases	Experimental status	Experimental result	References
HIF-1	Upregulation of PLK4 and induction of differentiation of M-MDSCs into TAM	NA	NA	NA	NA	NA	[16,74]
HiF-2	Reduces macrophage chemotaxis and M2-type polarization	Belzutifan	NCT04924075	Phase II	Recruiting	NA	[18]
HIF-3	RhoC-ROCK1 signaling pathway	NA	NA	NA	NA	NA	[19]
FAK	Substrate physical barrier	Defactinib	NCT02546531	Phase I	completed	Positive	[24]
HA	Adding IFP	NA	NA	NA	NA	NA	[37]
iCAFs	Activation of JAK-STAT pathway	Ruxolitinib	NCT02117479, NCT02119663, NCT02767557	Phase III, Phase II	completed	Negative	[49–53]
CSCs	Increased Oct4, Nanog, Sox2 expression, induction of EMT	NA	NA	NA	NA	NA	[59–61]
lncRNA	MACC1-AS1/PAX8/NOTCH1	NA	NA	NA	NA	NA	[65,66]
TAMs	Activation of TLR4/IL-10 pathway, M2-type polarization	PF-04136309	NCT01413022	Phase I/II	completed	Positive	[67–69]
NETs	Activation of IL-1 $\beta$ /EGFR/ERK pathway	NA	NA	NA	NA	NA	[80–82]
PD-1/PD-L1/CTLA-4	Immune response of Th1 lymphocytes	NA	NA	NA	NA	NA	[83,84]
CTGF	Phosphorylation of ERK	mAbFG-3019	NCT02210559	Phase II	completed	Positive	[92–94]
EGFR	Remodeling of tumor stroma, Activation of STAT3 pathway	Erlotinib	NCT02694536	Phase III	completed	Negative	[97,98]
IGF-1R	Activation of PI3K/Akt, mTOR, MEK/ERK pathway	MK-0646	NCT00769483	Phase I/II	completed	Positive	[100–104]
CXCL12/CXCR4	Activation of FAK, ERK and Akt pathways, Promotion of $\beta$ -catenin, NF- $\kappa$ B pathway	motixafortide	NCT02826486	Phase II	completed	Positive	[108–112]

CSC, cancer stem cells; CTGF, connective tissue growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FAK, focal adhesion kinase; HA, hyaluronan; iCAF, inflammatory cancer-associated fibroblast; IGF-1R, insulin-like growth factor 1 receptor; lncRNA, long non-coding RNA; MDSC, myeloid-derived suppressor cells; NET, neutrophil extracellular trap; TAM, tumor-associated macrophages; NA, not available.

Targeted anti-EGFR molecular strategies have been used to reverse the resistance of PDAC to GEM [95]. Among them, erlotinib is one of the most studied EGFR inhibitors. An earlier phase III clinical study showed that erlotinib combined with GEM treatment prolonged the overall survival of patients with pancreatic cancer [96]. Mechanistically, targeted inhibition of EGFR blocks STAT3 pathway activation, remodels the tumor stroma, increases microvascular density, and enhances GEM efficacy [97]. Unfortunately, a recent multicenter phase III clinical study (NCT02694536) found no notable difference in disease-free and overall survival for erlotinib combined with GEM compared with GEM monotherapy for advanced PDAC [98]. Additionally, c-Kit, an important member of the tyrosine kinase receptor protein family, is overexpressed in PDAC, and masitinib is a novel specific c-Kit inhibitor. Masitinib reverses the efficacy of PDAC against GEM and improves patient prognosis [99]. The IGF-1/IGF-1R pathway is upregulated in approximately 72% of patients with PDAC [100], promoting PDAC proliferation and inducing GEM resistance by activating the downstream PI3K/Akt/mTOR and MEK/ERK pathways [101]. Moreover, ganitumab specifically inhibits IGF-1R, and an earlier phase II clinical study showed that ganitumab combined with GEM prolonged the overall survival of PDAC with a favorable safety profile [102]. Unfortunately, subsequent phase III studies showed no positive results [103]. Notably, a recent phase I/II clinical trial in PDAC (NCT00769483) has demonstrated that the (MK-0646) IGF-1R inhibitor in combination with GEM + erlotinib considerably improved survival compared to GEM + erlotinib [104]. Reassuringly, a recent phase Ib study showed better results with dovitinib in combination with GEM in treating patients with advanced pancreatic cancer [105]. Dovitinib (TKI258) is a novel multi-targeted tyrosine kinase inhibitor (TKI) targeting FGFRs, PLT-derived growth factor receptor  $\beta$ , and vascular endothelial growth factor receptor-2 by modulating the Akt/Mcl-1 axis to promote apoptosis in tumor cells [106]. Similarly, tanshinone IIA (TanIIA; C19H18O3) enhanced GEM sensitivity by blocking Ras/Raf/MERK/ERK and PI3K/AKT/mTOR signaling pathways by downregulating EGFR, IGF1R, and VEGFR expression [107]. Therefore, multi-targeted TKI inhibitors combined with GEM and/or immunosuppressants are promising future strategies for a comprehensive treatment of PDAC.

#### 4.2 Chemokines

The CXC chemokine family (CXCL1-17) are small secreted proteins that induce GEM resistance by binding to their receptors (CXC chemokine receptor, CXCR) to regulate paracrine and autocrine pathways in PDAC mesenchymal cells that lead to GEM resistance [108,109]. A typical example is the CXCL12/CXCR4 axis that activates FAK, ERK, and Akt pathways to promote survivin expression, thus, inducing GEM resistance [110,111]. A phase IIa clin-

ical study (NCT02826486) showed that the CXCR4 antagonist BL-8040 (motixafortide) in combination with pembrolizumab prolonged the overall survival of patients [8]. Notably, in PDAC cells, artemin can induce CXCR4 overexpression by activating ERK1/2 and Akt pathways and further through NF- $\kappa$ B. Therefore, targeted inhibition of artemin may provide new therapeutic ideas for chemotherapy sensitization [112].

## 5. Conclusions and Prospects

In the past decade, there has been little improvement in the overall survival rate of PDAC, and the primary treatment regimen is still GEM-based chemotherapy. Overcoming chemoresistance is a key aspect of improving prognosis. Although current clinical trials targeting TME stromal components have yielded unsatisfactory results, research on the TME continues to advance, and clinical trials combining GEM with multiple immunotherapy targets have shown encouraging results, although previous immune monotherapies have mostly been ineffective [113] (Table 1, Ref. [16,18,19,24,37,49–53,59–61,65–69,74,80–84,92–94,97,98,100–104,108–112]). In addition to immunotherapy, targeted therapies, gene editing, microbial therapies, and combination therapies have exhibited promising results in the clinical management of PDAC. It is crucial to highlight that while these cutting-edge treatments hold immense potential for PDAC patients, many of them are still undergoing early-stage clinical trials, and need further research and validation to establish broader clinical applicability.

## Abbreviations

PDAC, pancreatic ductal adenocarcinoma; GEM, gemcitabine; TME, tumor microenvironment; PD-1, programmed death-1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T lymphocyte associated antigen-4; HIF, hypoxia-inducible factor; CA, carbonic anhydrase; PLK4, polo-like kinase4; CSCs, cancer stem cells; RhoC, Ras homolog family member C; ROCK1, Rho-associated coiled-coil containing protein kinase 1; FAK, focal adhesion kinase; MT1-MMP, membrane type-1 matrix metalloproteinase; HMGA2, high mobility group protein A2; LM, Laminin; TG2, transglutaminase 2; NF- $\kappa$ B, nuclear factor kappa B; aPSCs, Activated pancreatic stellate cells; CAFs, cancer-associated fibroblasts; IL-1, interleukin-1; EMT, epithelial–mesenchymal transition; LncRNA, long non-coding RNA; TAMs, tumor-associated macrophages; MD-SCs, Myeloid-derived suppressor cells; CXCL, chemokine ligands; CXCR, chemokine receptors; TILs, Tumor-infiltrating lymphocytes; PLTs, platelets; CTGF, Connective tissue growth factor; EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; IGF-1R, insulin-like growth factor 1 receptor; M-MDSCs, monocytic-myeloid-derived suppressor cells; MSCs, mesenchymal stem cells.

## Author Contributions

ZTG and RT proposed the research topic of the article, TPZ and RN collected the literature and wrote the paper. XW and QTJ performed the image design and revision. All authors participated in the editorial revision of the manuscript. All authors read and approved the final manuscript. All authors participated fully in the work, assumed public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work for issues related to the accuracy or completeness of the work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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