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REVIEW

Treatment resistance in pancreatic and biliary tract cancer: molecular and clinical pharmacology perspectives

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ABSTRACT

Introduction: Treatment resistance poses a significant obstacle in oncology, especially in biliary tract cancer (BTC) and pancreatic cancer (PC). Current therapeutic options include chemotherapy, targeted therapy, and immunotherapy. Resistance to these treatments may arise due to diverse molecular mechanisms, such as genetic and epigenetic modifications, altered drug metabolism and efflux, and changes in the tumor microenvironment. Identifying and overcoming these mechanisms is a major focus of research: strategies being explored include combination therapies, modulation of the tumor microenvironment, and personalized approaches.

Areas covered: We provide a current overview and discussion of the most relevant mechanisms of resistance to chemotherapy, target therapy, and immunotherapy in both BTC and PC. Furthermore, we compare the different strategies that are being implemented to overcome these obstacles.

Expert opinion: So far there is no unified theory on drug resistance and progress is limited. To overcome this issue, individualized patient approaches, possibly through liquid biopsies or single-cell transcriptome studies, are suggested, along with the potential use of artificial intelligence, to guide effective treatment strategies. Furthermore, we provide insights into what we consider the most promising areas of research, and we speculate on the future of managing treatment resistance to improve patient outcomes.

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Biliary tract cancer; chemoresistance; chemotherapy; immunoresistance; immunotherapy; pancreatic cancer; resistance mechanisms; target therapy

1. Introduction and current therapeutic options

The term, biliary tract cancers (BTCs), refers to malignancies that arise in the bile ducts, including intrahepatic bile ducts, extrahepatic bile ducts, and the gallbladder. Overall, BTCs are relatively rare compared to other cancers, accounting for approximately 3% of all gastrointestinal malignancies, but with various incidence rates around the world [1]. In the United States, the estimated annual incidence of BTC is around 2,500 to 3,000 cases [2]. The risk factors for BTC include chronic inflammation of the bile ducts (such as in primary sclerosing cholangitis), gallstones, liver fluke infections, and genetic alterations [1].

Pancreatic cancer (PC) is a highly aggressive malignancy that arises in the tissues of the pancreas. It is more common than BTC, being the 12th most common cancer worldwide and accounting for approximately 2% of all new cancer cases [3]. In the United States, PC is the 10th most common cancer, and it is the third leading cause of cancer-related deaths, having an estimated annual incidence of around 60,000 cases. The risk factors for PC include smoking, obesity, chronic pancreatitis, diabetes, family history of PC, as well as some genetic alterations [4].

1.1. Treatment algorithm in biliary tract cancer (BTC)

Despite the rarity of this disease, recent years have witnessed a substantial shift in the treatment paradigm of advanced BTC, with the introduction of both immunotherapy and target therapies (Table 1). Following the results of the phase-3 TOPAZ-1 trial, standard first-line treatment has become the combination of immune-chemotherapy with anti-programmed death-ligand 1 (PD-L1) durvalumab plus

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Article highlights

- Therapeutic options for BTC and PC include chemotherapy, targeted therapy, and immunotherapy, however the prognosis of these patients remains poor.
- Resistance to these treatments is common and it may arise from diverse molecular mechanisms, such as genetic alterations, epigenetic modifications, altered drug metabolism and efflux, and changes in the tumor microenvironment. Identifying and overcoming these mechanisms is a major focus of research.
- Addressing each patient individually, utilizing tools such as liquid biopsy or single-cell transcriptome studies can help identify primary mechanisms of resistance, allowing for targeted treatments.
- The article explores the emerging possibility of using artificial intelligence to integrate data on different treatment resistance mechanisms, aiming for a unified understanding and guiding the development of more effective strategies.
- Examples of success in personalized medicine, such as the development of new generation FGFR inhibitors in BTC and maintenance therapy with PARP inhibitor olaparib in BRCA1/2 mutated PC patients, are acknowledged.

cisplatin – gemcitabine (GEM) [5]. While the overall survival (OS) did not exhibit a substantial numerical increase (12.9 vs 11.3 months at the latest interim analysis), around 26% of patients in the immune-chemotherapy arm had a continued response after 1 year vs only 15% in the chemotherapy arm, showing how a long-lasting response can be obtained more frequently with immuno-chemotherapy [6]. Similar OS results have been observed with the combination of anti PD-1 pembrolizumab plus cisplatin-GEM, with an estimated ongoing response rate at 24 months of 18% [7].

Second-line treatment is now divided according to the presence of targetable mutations. Fibroblast growth factor receptor 2 (FGFR2) rearrangements have three Food and Drug Administration (FDA)-approved target drugs, pemigatinib, futibatinib, and infigratinib [8–11]. Although efficacy varies with each drug, OS is overall significantly longer (up to 21.7 months with futibatinib), and duration of response can range from 5 months with infigratinib, up to 9.1 and 9.7 months with pemigatinib and futibatinib, respectively. IDH1 mutations can be targeted using FDA-approved ivosidenib, based on a small progression-free survival (PFS) gain against placebo (2.7 vs 1.4 months) and PFS rates at 12 months of 22% vs 0%, although overall response rate (ORR) was only 2% [12]. Anti-Her2 therapy with zanidatamab was given FDA Breakthrough Approval in 2020 and the recent results of the HERIZON-BTC-01 trial are encouraging, with an ORR of 41% and PFS of 5.5 months [13]. Notwithstanding the agnostic therapies available for alteration such as neurotrophic tyrosine receptor kinase (NTRK), BRAF, RET, and high microsatellite instability (MSI-H), many other drugs are in the early stages of testing in BTC. This suggests the potential for an expansion in approved targeted treatments for BTC in the near future [14,15] (Table 1).

Second-line standard of care treatment for patients with no targetable mutations is still chemotherapy, with no clear preferred treatments. One of the most common regimens, oxaliplatin–5-fluorouracil is associated with a small but statistically significant OS gain when compared to active symptom control (6.2 vs 5.3 months), but with poor ORR (5%) and disease control rate (33%), indicating high levels of primary resistance [16]. A novel option is the use of liposomal-irinotecan +5-fluorouracil, associated with PFS ranging from 2.7 to 7.1 months, and ORR of around 15% [17,18]. Several chemotherapy drugs have been experimented within second or subsequent lines of therapy, yet none has demonstrated a clear advantage over the others [19] (Table 1).

1.2. Treatment algorithm in pancreatic cancer (PC)

Chemotherapy with conventional anticancer agents is still the first-line treatment standard in metastatic PC (Table 2). The most commonly used regimens are FOLFIRINOX (5-fluorouracil + irinotecan + oxaliplatin) or GEM + nab-paclitaxel, both with an OS of less than 1 year (11 and 8.5 months respectively), ORR between 31.6% and 23%, and PFS rates at 12 months of 12% and 16% [20,21]. A similar 11.1 months OS result has been observed with the NALIRIFOX regimen (5-fluorouracil, liposomal irinotecan, oxaliplatin), although with a better PFS rate at 12 months of 27% vs 14% of the GEM + nab-paclitaxel combination [22].

When it comes to second or subsequent lines of therapy, the foundation remains chemotherapy-based, with many options available and no clear advantage of one over the others. Thus, treatment is usually personalized according to patients' performance status, expected toxicities, and previous treatments. OS is usually between 3 and 9 months, and ORR varies between 10% and 20% for combination treatments to less than 10 for the monotherapies arm [23–26] (Table 2).

Regarding novel approaches, target therapies have not yielded significant success in the context of PC. There is, however, an exception for maintenance therapy with poly (ADP-ribose) polymerase (PARP) inhibitor, olaparib, in the setting of BRCA1–2 germ-line mutated patients who have not

Table 1. Treatment regimens in BTC.

Line	Regimen	Phase	Number of patients enrolled	Median OS (months)	Authors	Year
First-line	PD-1 Durvalumab + Cisplatin + GEM	III	685	11.7	Oh DY et al. [5]	2022
First-line	PD-1 Pembrolizumab + Cisplatin + GEM	III	1564	12.7	Kelley RK et al. [7]	2023
Second-line	Pemigatinib	II	146	17.8	Abou-Alfa GK et al. [8]	2020
Second-line	Pemigatinib	II	147	17.5	Vogel A et al. [9]	2022
Second-line	Futibatinib	II	103	21.7	Goyal L et al. [10]	2023
Second-line	Infigratinib	II	122	10.6	Javle M et al. [11]	2021
Second-line	Futibatinib	III	290	6.2	Lamarca A et al. [16]	2021
Second-line	Liposomal-Irinotecan +5FU	II	193	7.1	Yoo C et al. [17]	2021
Second-line	Liposomal-Irinotecan +5FU + LV	II	100	6.9	Vogel A et al. [18]	2022

Abbreviations: N: number of patients, OS: overall survival, PD: programmed cell death protein, GEM: gemcitabine, 5FU: fluorouracil, LV: leucovorin.

Table 2. Treatment regimens in PC.

Line	Regimen	Phase	Number of patients enrolled	Median OS (months)	Authors	Year
First line	5FU + Irinotecan + Oxaliplatin	II-III	342	11.1	Conroy T et al. [20]	2011
First line	GEM + Nab-paclitaxel	I-II	861	8.5	Von Hoff D et al. [21]	2013
First line	5FU + Liposomal Irinotecan + Oxaliplatin	III	770	11.1	Wainberg Z et al. [22]	2023
Second line	GEM + Nab-paclitaxel	II-III	57	8.8	Portal A et al. [23]	2015
Second line	Folfirinox + Irinotecan + Oxiplatin	II	48	9	Chung M et al. [24]	2018
Second line	5FU/Leucovorin (+ Oxaliplatin)	III	108	6.1	Gill S et al. [25]	2016
Second line	Nanoliposomal Irinotecan + 5FU + Folinic Acid	III	417	6.1	Wang-Gillam A et al. [26]	2016
Second line	Olaparib	III	154	18.9	Golan T et al. [27]	2019
Second line	Erlotinib + GEM	III	569	6.24	Moore M et al. [28]	2007
Second line	Entrectinib	I-II	54	10	Doebele R et al. [29]	2020
Second line	Larotrectinib	I-II	159	44.4	Hong D et al. [30]	2020
Second line	Pembrolizumab	II	233	23.5	Marabelle A et al. [31]	2020

Abbreviations: N: number of patients, OS: overall survival, 5FU: fluorouracil, GEM: gemcitabine, PEGPH20: PEGylated recombinant human hyaluronidase, mFOLFIRINOX: oxaliplatin, leucovorin, irinotecan, and fluorouracil.

progressed during first-line platinum-based chemotherapy: this therapy was associated with longer PFS (7.4 vs 3.8 months in the placebo arm) and a doubled PFS rate at 12 months (33.7 vs 14.5%), however, this advantage did not translate into an OS gain [27]. Epidermal growth factor receptor (EGFR) inhibitor erlotinib has been tested alongside GEM vs GEM monotherapy, obtaining a 3 weeks gain in OS and similar ORR, thus this combo did not see much application in clinical practice [28]. Although the rate of PCs with genomic alterations susceptible to agnostic therapies is very low, Tyrosine Kinase Inhibitor (TKI) treatment for NTRK fusion-positive cases and immunotherapy for cases with MSI-H can sometimes be applied [29–31]. Many other targets are or have been tested, but none of them are being used outside of clinical trials [32] (Table 2).

2. Molecular mechanisms of chemoresistance

In the field of medical research, gaining a deep understanding of the intricate molecular mechanisms underlying chemoresistance is of the utmost importance. Within the intricate web of cellular processes, various molecular players collaborate to orchestrate resistance, diminishing the efficacy of conventional treatments [33]. Understanding these mechanisms holds great significance in the development of precision therapies that can overcome chemoresistance.

2.1. Chemoresistance in BTC

Despite advances in diagnostic techniques and therapeutic modalities, the prognosis for patients with BTC remains bleak, primarily due to the high incidence of chemoresistance. Here, we review the current knowledge regarding the molecular mechanisms contributing to chemoresistance in BTC (Figure 1).

2.1.1. Genetic alterations

Genetic alterations play a pivotal role in the development of chemoresistance to various chemotherapeutic agents. However, preclinical studies regarding the role of different genetic aberrations in chemoresistance and their 'targeting' in BTC models are limited [34] (Figure 1).

A study in both intra- and extra-hepatic cholangiocarcinoma (ICC and ECC) cells demonstrated that enhancement of expression of the tumor suppressor gene *TP53*, resulted in the up-regulation of p21 and a reduction in cell proliferation [35]. Furthermore, these models showed up-regulation of protumoral proteins FAS, BAX, TYMP, and CES2, coupled with down-regulation of DHFR, RRM1, and BIRC5. These modifications were accompanied by increased sensitivity to antitumor drugs, particularly platinated drugs. Similar results were observed in ICC cells KKU-100 and KKU-M214, tested for their sensitivity to 5-fluorouracil, doxorubicin, and GEM [36]. Enhanced chemosensitivity to all these anticancer drugs was attributed to the activation of p53-mediated cell death. Indeed, enhanced susceptibility to chemotherapeutic agents by the antioxidant/detoxifying enzyme NAD(P)H-quinone oxidoreductase-1 was abolished by knockdown of *TP53*, hinting to an important role of p53 and its alterations in the development of chemoresistance.

Dysregulation of oncogenes, such as the *KRAS*, might also contribute to chemoresistance by promoting cell survival and inhibiting apoptosis (Figure 1). Gain-of-function mutations in *KRAS* are present in approximately 45–55% of ICC and 10–15% of ECC. Furthermore, one study showed that *BRAF*, an important downstream effector of *KRAS*, was mutated in up to 22% of ICC cases [37].

Of note, Peng et al. (2023) investigated the correlation between *TP53* and *KRAS* in cholangiocarcinoma (CCA) patients, showing that these alterations were significantly associated with shorter PFS [38]. Thus, aberrations of *TP53* and *KRAS* could serve as predictive indicators of chemoresistance and of an unfavorable prognosis in CCA patients (Figure 1).

Interestingly, a recent study showed that the tyrosine phosphatase SHP2 directly governs the activity of the transcription regulator protein YAP by dephosphorylating it and establishing an association between reduced SHP2 phosphatase activity and chemoresistance in CCA cells, even in the context of *RAS/RAF* mutations [39]. Cell lines characterized by low SHP2 expression and elevated phosphorylated form of YAP were resistant to GEM and cisplatin. Moreover, the marked chemoresistance of xenografts with genetically deleted SHP2 was overcome by using an inhibitor directed against YAP target genes, such as the antiapoptotic regulator MCL1.

Regarding relatively common mutations such as *FGFR* alterations, although data suggest a positive prognostic role, no correlation with response to first-line therapy has been found [40,41]. Conversely, alterations in DNA repair genes, such as *BRCA1* and *BRCA2*, have been linked to higher

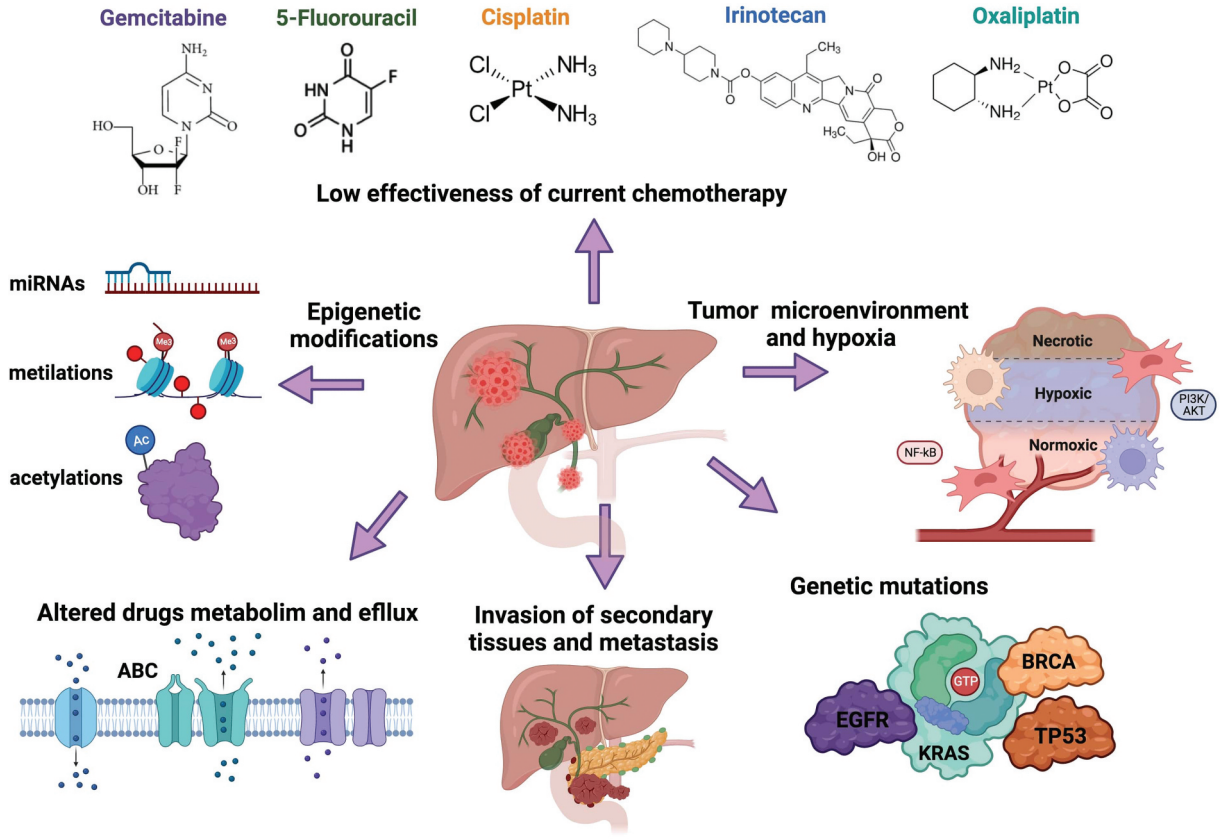

Figure 1. Molecular mechanisms of chemoresistance in BTC. chemoresistance in biliary tract cancer is mainly caused by epigenetic modifications, genetic mutations, hypoxic tumor microenvironment, great metastatic capacity of tumor cells and deregulation of metabolic pathways induced by chemotherapeutic agents, including changes in drug entry and exit transporters and changes in enzymes involved in drug effects. This is supported by the effective reduction of current cytotoxic drugs. Created with Biorender.com.

sensitivity to platinum-based chemotherapy (Figure 1). Kim et al. investigated the correlation between DNA repair gene mutations and the clinical response to platinum-based chemotherapy in patients with BTC and found that *BRCA* mutations were significantly associated with PFS at the multivariate analysis (HR 0.150, 95% CI: 0.034–0.655, $p = 0.012$) [42]. This study demonstrated that *BRCA* mutations might have a role as predictive biomarkers for first-line platinum-based chemotherapy in patients with advanced BTC. Of note, a study in three CCA cell lines (QBC939, HuH28 and TFK-1), showed that the radiosensitivity of CCA cells was enhanced by PARP inhibitor olaparib, inducing DNA lesions and apoptosis [43]. These findings hint to a strong relationship between gene alteration and response to therapy and spark the debate on overcoming resistance to traditional anticancer drugs by using synergistic combinations with novel targeted drugs (Figure 1).

2.1.2. Epigenetic modifications

Epigenetic modifications, including DNA methylation, histone modifications, and non-coding RNA expression, have emerged as critical regulators of BTC pathogenesis (Figure 1).

CCA tissue exhibits a significant decrease in DNA hydroxymethylation compared to non-tumor tissue [44]. Additionally, hyper-methylation is observed in several gene promoters related to Wnt signaling, including the P16INK4a gene, found in up to 83% of CCA cases. P16INK4a inhibits the interaction between cyclin D1 and cyclin-dependent kinase 4 (CDK4). Methylation of the promoter halts P16INK4a activity, allowing CDK4 to bind to cyclin D1, initiating the cell's entry into the S phase. The methylation of the CpG island is the primary factor leading to P16INK4a inactivation [45]. Treatment with CDK4/6 inhibitors in 15 CCA cell lines resulted in decreased number of cells in S-phase and induced senescence. The efficacy of this treatment was further confirmed in spheroids and patient-derived xenografts [46].

Furthermore, previous studies have highlighted the involvement of miRNAs in chemoresistance [47] (Figure 1). For example, Meng et al. demonstrated that miR-200b and miR-21 play roles in sensitivity to GEM, with their inhibition significantly increasing GEM cytotoxicity and apoptotic effects in CCA cell lines [48]. Inhibition of miR-200b correlated with increased expression of protein phosphatase non-receptor type 12, impacting cell proliferation and differentiation, while inhibition of miR-21 led to decreased expression of its direct target, PTEN, influencing the PI-3 kinase pathway and promoting cell survival.

A study on the expression of 2555 miRNAs in CCA cells observed deregulation of 137 and 14 miRNAs in GEM-treated HuCCT-1 and TKKK cell lines, respectively, in comparison to their untreated controls [49]. Specifically, miR-664b-3p, miR-3651, and miR-6087 exhibited increased expression in HuCCT-1 cell lines but were downregulated in TKKK cell lines, suggesting a potential role for these miRNAs in influencing the sensitivity of CCA cells to GEM [49].

More recently, a high-throughput screening of 997 locked nucleic acid miRNA inhibitors in 6 CCA cell lines treated with cisplatin and GEM revealed that miR-1249 inhibition enhanced chemotherapy sensitivity across all tested cells [50]. miR-1249 was found to be upregulated in CD133+ cells from human BTC stem cell niches and in chemo-resistant CCLP cells. Knockout of miR-1249 resulted in impaired expansion and enrichment of CD133+ subclones, reduced expression of cancer stem cell markers, and increased chemosensitivity. In xenograft models, miR-1249 knockout led to tumor shrinkage after exposure to weekly cisplatin and GEM, while wild-type models exhibited stable disease over treatment. Moreover, overexpression of miR-1249 was present in 41% of human BTCs cases, suggesting its potential both as biomarker of chemoresistance and as a potential target.

2.1.3. Tumor microenvironment (TME)

TME plays a crucial role in chemoresistance by providing a supportive niche for cancer cells (Figure 1). Stromal cells, including cancer-associated fibroblasts (CAFs) and immune cells, secrete various factors, such as Transforming Growth Factor beta (TGF-β) and interleukins, that promote tumor growth and survival [51] (Figure 1). These factors can activate signaling pathways, such as PI3K/AKT and NF-κB, which contribute to chemoresistance [52]. In particular, a recent study by Obata et al. (2023) demonstrated that this activation contributes to GEM resistance in biliary tract cancer cells, promoting cell survival and suppressing apoptosis [53]. The study proposed miR-451a as a potential therapeutic target in gallbladder cancer (GBC), because it significantly impeded cell proliferation, induced apoptosis, and mitigated chemoresistant features, including epithelial–mesenchymal transition (EMT), in both GBC and GEM-resistant GBC. This effect was likely mediated through the negative regulation of the phosphatidylinositol 3-kinase/AKT pathway, achieved in part by directly downregulating macrophage migration inhibitory factor [53].

In a separate study conducted by Yang et al., it was demonstrated that the NF-κB pathway plays a crucial role in the emergence of chemoresistance in biliary tract cancer (BTC) cells. GEM-resistant gallbladder cancer (GBC) cells exhibited low expression of KRAS and inactivation of AKT/ERK signaling. Conversely, in the same resistant cells the p70S6K, p38MAPK, and NF-κB signaling pathways were activated [54].

Moreover, the hypoxic and nutrient-deprived conditions within TME can induce a quiescent state in cancer cells, rendering them less susceptible to chemotherapy [55] (Figure 1). Interestingly, the hypoxia-induced gene 2-oxoglutarate 5-dioxygenase 2 (PLOD2), was upregulated in BTC cells resistant to GEM, which were also characterized by low expression of epithelial markers and high expression of mesenchymal markers. The use of siRNA to downregulate PLOD2 led to reduction of chemoresistance, restoration of epithelial markers, and decrease of mesenchymal markers. In resected BTC samples, PLOD2 expression showed a significant correlation with lymph node metastasis and stage of the disease [55]. Moreover, patients with high PLOD2 expression exhibited significantly lower recurrence-free survival and OS rates. Considering these findings, PLOD2 could be explored as a potential prognostic biomarker and therapeutic target for overcoming chemoresistance.

Collectively, we highlight the well-studied multifaceted nature of chemoresistance in BTC, urging further exploration of targeted therapies and combination strategies to enhance treatment efficacy and improve patient outcomes in this challenging disease.

2.2. Chemoresistance in PC

PC stands as one of the most formidable challenges in modern oncology, characterized by its aggressive nature and limited treatment success [56,57]. While significant strides have been made in understanding the molecular underpinnings of this malignancy, therapeutic progress has been impeded by the common inherent and acquired chemoresistance [58]. This review seeks to provide an overview of the existing knowledge surrounding the molecular mechanisms implicated in chemoresistance in PC (Figure 2).

2.2.1. Genetic alterations

Genetic alterations play a crucial role in the development of chemoresistance in PC. For instance, mutations in *KRAS*, which are detected in more than 90% of PC patients, have been associated with resistance to multiple chemotherapeutic agents (Figure 2). Certain studies have also suggested that targeting *KRAS* may lead to the attenuation of chemoresistance and improve the therapeutic response in PC patients. Recent preclinical studies have shown that the use of SHP2 inhibitors (SHP099), MEK inhibitors (cobimetinib), and KRAS-G12C inhibitors (sotorasib), can enhance the efficacy of chemotherapeutic agents in PC cells carrying *KRAS* mutations, as well as an acceptable safety profile in pretreated patients with KRAS-mutated advanced PC [59–61].

Alterations in *TP53*, have also been identified as key factors contributing to this resistance (Figure 2). Pan and colleagues demonstrated that the loss of p53 function has been associated with aggressive tumor phenotypes, chemoresistance, and poor prognosis in PC patients, involving several mechanisms, such as dysregulation of cell cycle arrest, DNA damage repair, apoptosis, and autophagy [62].

Mutations that impact genes responsible for repairing DNA damage, such as *BRCA1/2*, *ATM*, and *PALB2*, have been extensively studied for their role in response to chemotherapy [63,64] (Figure 2). These genes encode important proteins involved in homology-directed repair (HDR), which is a system for accurately fixing DNA double-strand breaks (DSB) [65]. When there are mutations that disrupt the function of these genes, HDR is impaired, and cells rely more on error-prone repair mechanisms like non-homologous end joining and other DSB repair systems [66]. In 2018, Blair et al. found that a group of PC patients with *BRCA1/2* mutations had better outcomes when treated with platinum-based adjuvant chemotherapy compared to non-platinum-based adjuvant chemotherapy or nothing [67]. Additionally, a retrospective study on 262 patients, of which 50 had HDR mutations, showed that HDR-mutated patients had better PFS when treated with first-line platinum therapy [68]. Furthermore, a recent phase II study demonstrated that GEM + cisplatin treatment is effective against PCs with both *BRCA1/2* and/or *PALB2* mutations [69].

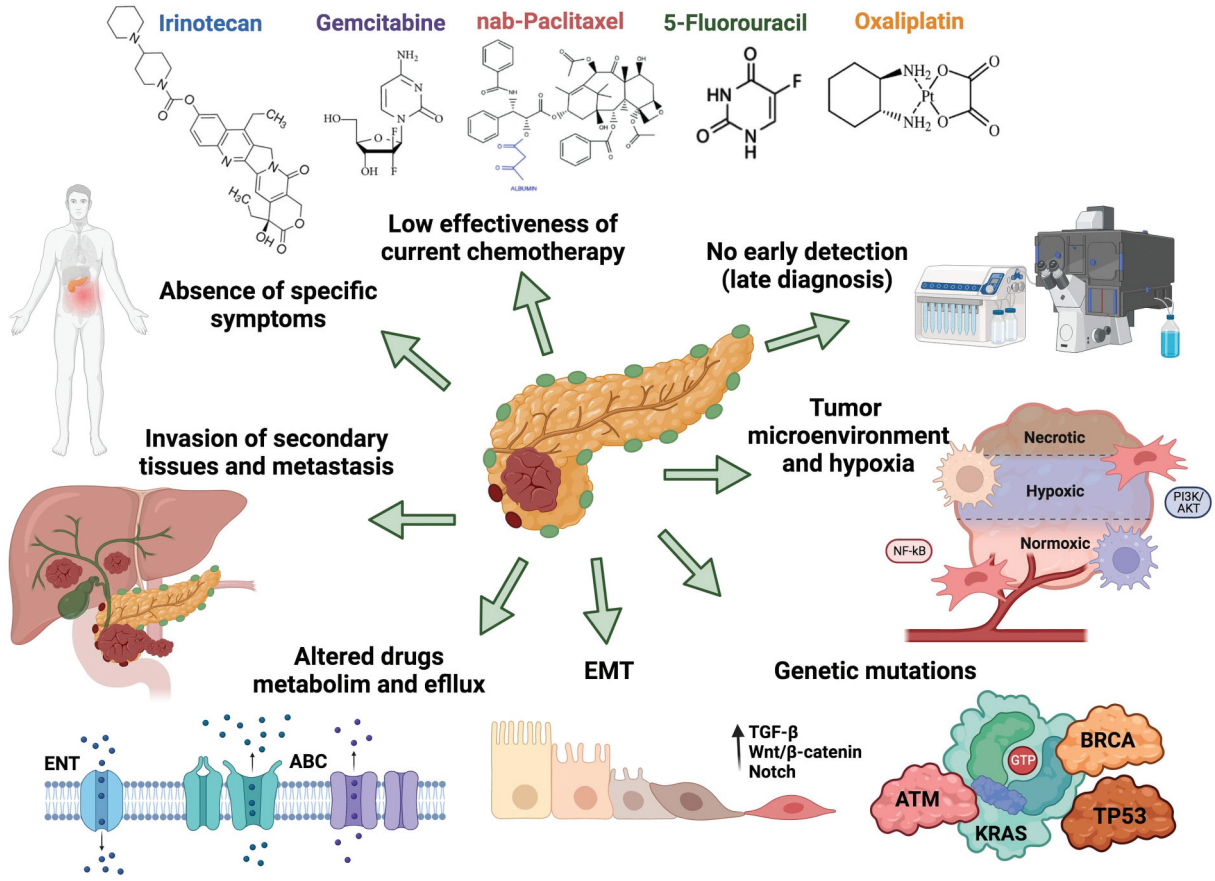

Figure 2. Molecular mechanisms of chemoresistance in PC. chemoresistance in pancreatic cancer is mainly contributed by the epithelial–mesenchymal transition phenotype, genetic modifications, the hypoxic tumor microenvironment, the great metastatic capacity of tumor cells and the deregulation of metabolic pathways induced by chemotherapeutic agents, including changes in drug entry and exit transporters and changes in enzymes involved in drug effects. This is supported by the factors of an absence of symptoms that allow early detection and diagnosis, as well as the effective reduction of current cytotoxic drugs. Created with Biorender.com.

2.2.2. Epigenetic modifications

Several epigenetic modifications, such as DNA methylation, histone acetylation and deacetylation, as well as histone methylation have been implicated in chemoresistance in PC. Most of these studies have demonstrated that disrupting DNA methylation homeostasis is a key factor in the development of human cancer. Furthermore, these studies have contributed to the realization that alterations in methylation patterns play a crucial role in distinguishing tumor cells and rendering them resistant to chemotherapy [70]. The abnormal methylation patterns in PC have been extensively described [71] (Figure 2).

Aberrant expression of noncoding RNAs, such as microRNAs and long noncoding RNAs, can also impact the response to chemotherapy as they can act as oncogenes or tumor suppressor genes [72–74] (Figure 2). Hong and colleagues found that miR-21, miR-27a, miRNA-146a, miRNA-196a, and miRNA200a were the most upregulated miRNAs, while miR-20a, miR-96, and miR-217 were significantly downregulated in PC tissues [75,76]. This suggests that some miRNAs could promote oncogenic processes while others have tumor-suppressive effects. Several studies showed the role of miR-21 in chemoresistance and poor prognosis [77–79], and the involvement of other miRNAs has been recently reviewed by Vahabi and collaborators [80].

2.2.3. Epithelial–mesenchymal transition (EMT)

EMT is a critical process involved in the aggressive nature of cancers, leading to invasion, metastasis, and drug resistance [81] (Figure 2). El Amrani et al. conducted a study revealing that GEM induces EMT-like changes in PC cells [82]. These changes are mediated by the extracellular regulated kinase (ERK)-zinc finger E-box binding homeobox 1 (ZEB1) pathway. Inhibition of ERK1/2 phosphorylation or ZEB1 expression leads to a reduction in chemoresistance and invasion of GEM-resistant (GR) PANC-1 and MIAPaCa-2 cells. Additionally, GEM induced overexpression of CD44, CD24, and CD326 in GR cells compared to sensitive cells. Further studies have identified that AMPK-related kinase 5 and upregulation of glycolysis contribute to GEM resistance through EMT mechanisms.

Kuwada et al. demonstrated that the induction of EMT in PANC-1 and BxPC-3 cells by tumor associated macrophages (TAMs) render them more migratory and thereby promote growth and chemoresistance [83].

Lastly, EMT is closely linked to the modulation of the TGF-β pathway which is a key regulator of autophagy and apoptosis [84]. The TGFβ-miR200-MIG6 pathway orchestrates a kinase switch linked to epithelial–mesenchymal transition (EMT), resulting in resistance to EGFR inhibitors [85]. Noteworthy the antipsychotic drug brexpiprazole is able to overcome osimertinib resistance in both lung cancer and PC by

suppressing survivin protein. This action subsequently inhibits TGF-β/SMAD signaling, preventing EMT [86,87]. The regulation of hMENA isoforms by TGF-β1 is pivotal in TGF-β1-induced EMT, presenting potential targets for the development of innovative prognostic and therapeutic strategies in PC [88].

2.2.4. Altered drug influx and efflux

Human equilibrative nucleoside transporter-1 (hENT-1 or SLC29A1) has been shown to play a crucial role in the intracellular accumulation of nucleoside-based chemotherapeutic agents in PC cells, especially GEM [89,90] (Figure 2). Previous studies reported that the downregulation/inhibition of hENT-1 resulted in cell chemoresistance to GEM and a retrospective analysis of the phase-3 clinical trials RTOG-9704 and ESPAC-1/3 supported the role of hENT1 as a predictive biomarker of GEM efficacy, with patients with high hENT-1 expression having a significantly longer OS [91–93]. Of note, this association was missing in patients treated with 5-FU, suggesting a predictive and not prognostic role for hENT-1.

Similarly, another transport called dENT-2 (SLC29A2) has been identified in various PC cell lines, although with decreased expression compared to hENT-1 [94]. It has been shown that h-ENT2 expression and its mRNA levels decrease after treatment with GEM in resistant cell lines [95]. Expanding on these findings, deactivation of both hENT-2 and hENT-1 results in diminished GEM uptake and sensitivity.

Efflux is the active process through which cells pump drugs out of their cytoplasm to avoid drug buildup. Thus, the expression of efflux pumps like ATP-binding cassette (ABC) multidrug transporters like ABCB1 have been found to play a role in drug resistance, drug distribution, and toxicity [96] (Figure 2). For instance, in the context of irinotecan, ABCB1 plays a role in the cellular uptake of both the prodrug and its active metabolite SN-38. However, the ABCB1 1236C >T variant markedly reduces irinotecan clearance, the ABCC2 3972T >C variant is linked to toxicity, and cells over-expressing ABCG2 are recognized as resistant to both irinotecan and SN-38 [97].

Chen and collaborators reported a significantly lower promoter methylation level of the AB transporter family (ABCB1, ABCC, and ABCG2) in a GEM-resistant cell line (SW1990/GZ) compared to primary cells [98]. Another study indicated a gradual decrease in the promoter methylation level of ABCB1, ABCC, and ABCG2 during the establishment of GEM resistance in PANC-1 and BxPC-3 cells [99]. This provides evidence of the theoretical feasibility of predicting multi-drug resistance (MDR) of PC through independent indicators such as the promoter methylation level of ABCB1, ABCC and ABCG2. However, clinical trials evaluating the predictive role of these transporters did not translate into successful clinical application.

Other interesting studies evaluated the expression and activity of drug-metabolizing enzymes. Recently, a study conducted by Yada et al. investigated the role of cytochrome P450 enzymes in chemoresistance of PC, showing that mRNA expression of cytochrome-P450 was upregulated in a concentration-dependent manner following GEM treatment. Moreover, the sensitivity to GEM increased with the use of a cytochrome-P450 inhibitor, indicating that this enzyme may be related to GEM resistance in PC [100].

The combination of nab-paclitaxel and GEM is another primary treatment option for patients diagnosed with advanced or metastatic PC [21]. Notably, the efflux of paclitaxel is facilitated by phosphorylated glycoprotein [101] and a recent study showed its key role in the resistance of SUIT02, PANC-1, and PaTu-T cells [102]. The glycoprotein known as secreted protein acidic and rich in cysteine (SPARC) has been extensively studied as a potential biomarker for assessing the activity of nab-paclitaxel, correlating it with PC cell proliferation and metastasis [103]. An initial immunohistochemical study conducted on 36 patients revealed a dose-dependent correlation between higher levels of SPARC expression and improved OS, however this was not conformed in following studies [104]. Furthermore, nab-paclitaxel has exhibited the ability to inactivate cytidine deaminase, the enzyme which mediates the conversion of GEM into its inactive form, thus resulting in increased levels of GEM and a higher response rate in KPC models [105].

2.2.5. Tumor microenvironment

Approximately 80% of PC tumor mass is constituted by stroma comprising cellular components (stellate cells, fibroblasts, endothelial and immune cells) and an extracellular matrix (ECM). Tumor stroma contributes to tumorigenesis by promoting invasion and enhancing tumoral angiogenesis, however the new vessels are often defective, explaining the hypoxic environment often observed in TME [106]. In addition, the stroma creates physical barriers that limit drug penetration and promotes drug resistance, by activating signaling pathways that are involved in cell proliferation [107,108] (Figure 2). Various growth factors (such as TGF-β, VEGF, CTGF, HGF, FGF), matricellular proteins, metalloproteinases, tissue inhibitors of metalloproteinases, and cytokines have been documented to impact drug effectiveness.

Sonic hedgehog (Shh), a soluble ligand, over-expressed by neoplastic cells in PC, drives formation of a fibroblast-rich desmoplastic stroma. Surprisingly, Shh-deficient tumors are more aggressive and exhibit undifferentiated histology, increased vascularity, and heightened proliferation features [109]. Together, these data demonstrate that some components of the tumor stroma can also act to restrain tumor growth and should not be inhibited.

Some stromal cells, including CAFs and immune cells, can play an active role in promoting chemoresistance. They provide cancer cells with survival signals, activate signaling pathways that facilitate drug efflux, and secrete cytokines that hinder apoptosis [110,111]. Lastly, the ECM, composed of proteoglycans, hyaluronic acid (HA), and collagen, plays a crucial role in influencing interstitial fluid pressure and blood vessel distribution, contributing to hypoperfusion, hypoxia, and altered cancer cell metabolism. This, in turn, reduces drug delivery and activity [112,113] (Figure 2).

In summary, PC presents one of the most formidable challenges in oncology, marked by its aggressive nature and limited treatment success, largely attributed to the pervasive issue of chemoresistance. This comprehensive review delves into the intricate molecular mechanisms underlying chemoresistance in PC, highlighting genetic alterations, epigenetic modifications, EMT, altered drug influx and efflux, and the TME as key contributors.

2.3. Overcoming chemoresistance

As seen before, the mechanisms underlying chemoresistance are complex, numerous, and often intertwined. Despite these difficulties, several attempts have been made to try to overcome chemoresistance in both BTC and PC (Table 3).

Given the genetic alterations associated with chemoresistance, one approach is to combine standard chemotherapy with drugs directed against genes involved in chemoresistance, and this subject is explored in the dedicated chapter on target therapy (Figure 3).

Regarding epigenetic changes that can lead to chemoresistance, Kurdistani et al. analyzed the role of histone modifications in GEM resistant PC [72]. The researchers evaluated the effects of histone deacetylase inhibitors (HDACi) on PC cell lines and patient-derived xenograft models. They found that treatment with the HDACi vorinostat sensitized cells to GEM, resulting in decreased cell viability and increased apoptosis. Analysis of histone modifications revealed that treatment with vorinostat led to increased acetylation of histones H3 and H4, as well as decreased methylation of histone H3 lysine 27, which is associated with gene silencing. Furthermore, combination treatment with vorinostat and GEM resulted in improved survival in PC xenografts compared to either treatment alone. A neoadjuvant phase 1 trial with the combination of vorinostat plus multi TKI sorafenib and chemoradiotherapy demonstrated good tolerability and encouraging antitumoral activity [114]. This suggests that modulation of histone modifications through HDACi holds potential for overcoming GEM-resistance in PC. Furthermore, DNA methyltransferase inhibitors have also being explored as epigenetic therapy: a phase 1 trial including both PC and BTC of guadecitabine and durvalumab was associated with a good tolerability profile and although the efficacy was limited, a group of patients had prolonged benefit, indicating the need for better biomarkers of response for this class of drugs [115].

Epigenetic changes can also take the form of alternation in miRNA, thus several studies have explored the idea of miRNA silencing or the use of miRNA mimics [116]. A new drug targeting the chemokine receptor CXCR4 along with miR-210 in CCA cell lines showed that this drug reversed the hypoxia-induced drug resistance and increased sensitivity to treatment with GEM plus cisplatin [117]. Overall treatment with miRNA has been widely explored in the preclinical setting, however a phase-1 trial on a drug targeting miR-34a in solid tumors including PC and CCA [118], showed that the treatment was associated with high infusion related adverse events.

Efforts to address phenotypic changes associated with chemoresistance due to EMT have been undertaken by targeting crucial pathways, albeit with limited success. For instance, drugs aimed at Notch, a pathway implicated in the emergence of CD44-positive PC stem cells and EMT in preclinical models, yielded unsatisfactory outcomes [119]. In a phase-1/2 clinical trial, the monoclonal antibody against Notch2/Notch3 (tarextumab) failed to enhance overall survival in combination with GEM and nab-paclitaxel. Moreover, the gamma-secretase inhibitor MK0752, when combined with GEM, resulted in severe adverse effects, causing a high rate of patient withdrawal [120,121] (Table 3).

Recent studies have indicated that metformin, a medication used for diabetes, enhances the antiproliferative effects of PC cells by inhibiting EMT. This effect might potentially improve the overall prognosis of PC patients. In fact, therapeutic exposure to metformin was associated with reduced morbidity and

Table 3. Overcoming chemoresistance in BTC and PC.

Tumor type	Target	Regimen	Results	Authors	Year
PC	Histone modifications	HDACi Vorinostat	– Viability decreased and apoptosis increased in cells – Combinations improved survival in PC xenografts	Kurdistani S.K et al. [72]	2004
CCA	Chemokine receptor CXCR4	miR-210	– Hypoxia reduced – Sensitivity to drug increased	Xie Y et al. [117]	2018
PC and CCA	Oncogenic pathways	MRX34 (liposomal miR-34a mimic)	– Acceptable safety – Evidence of antitumor activity in some patients	Beg M.S et al. [118]	2017
PC	Notch pathway	Tarextumab (Notch antagonist)	– Tumor growth inhibited – Tumor initiation cells frequenty decreased	Yen W. C et al. [119]	2017
PC	Notch pathway	Tarextumab + GEM + Nab-paclitaxel	– Similar OS in patients	O'Reilly E. M et al. [120]	2017
PC	Notch pathway	MK0752 + GEM	– Severe adverse effecs	Cook N et al. [121]	2014
PC	EMT	Metformin	– Cancer cells proliferation inhibited – Morbidity and mortility rates reduced – Short term survival rate slightly increased – No differences in long term survival rate	Gulla A et al. [122]	2022
PC	EMT	5-AZA	Tumor growth reduced – Less aggressive tumor model – More drug/sensitive tumor model	Gailhouste L et al. [125]	2018
PC	Hedgehog pathway	IPI-926	– No remarkable results	Jimeno A et al. [127]	2013
PC	Hyaluronic acid	PEGPH20 + GEM + Nab-paclitaxel	– PFS and OS was slightly increased	Hingorani S R et al. [128]	2018
PC	Hyaluronic acid	PEGPH20 + FOLFIRINOX	– Toxicity and secondary effects	Ramanathan R K. et al. [129]	2019
PC	JAK2/STAT3 pathway	Ruxolitinib + Capecitabine	– Well tolerated by patients – OS lightly increased	Hurwitz H. I et al. [130]	2015
PC	JAK2/STAT3 pathway	Ruxolitinib + Capecitabine	– Clinical results do not improve in second-line treatment	Hurwitz H. I et al. [131]	2017

Abbreviations: CCA: cholangiocarcinoma, PC: pancreatic cancer, EMT: epithelial-mesenchymal transition, miR: microRNA, GEM: gemcitabine, 5-AZA: 5-azacytidine, OS: overall survival, PFS: progression-free survival.

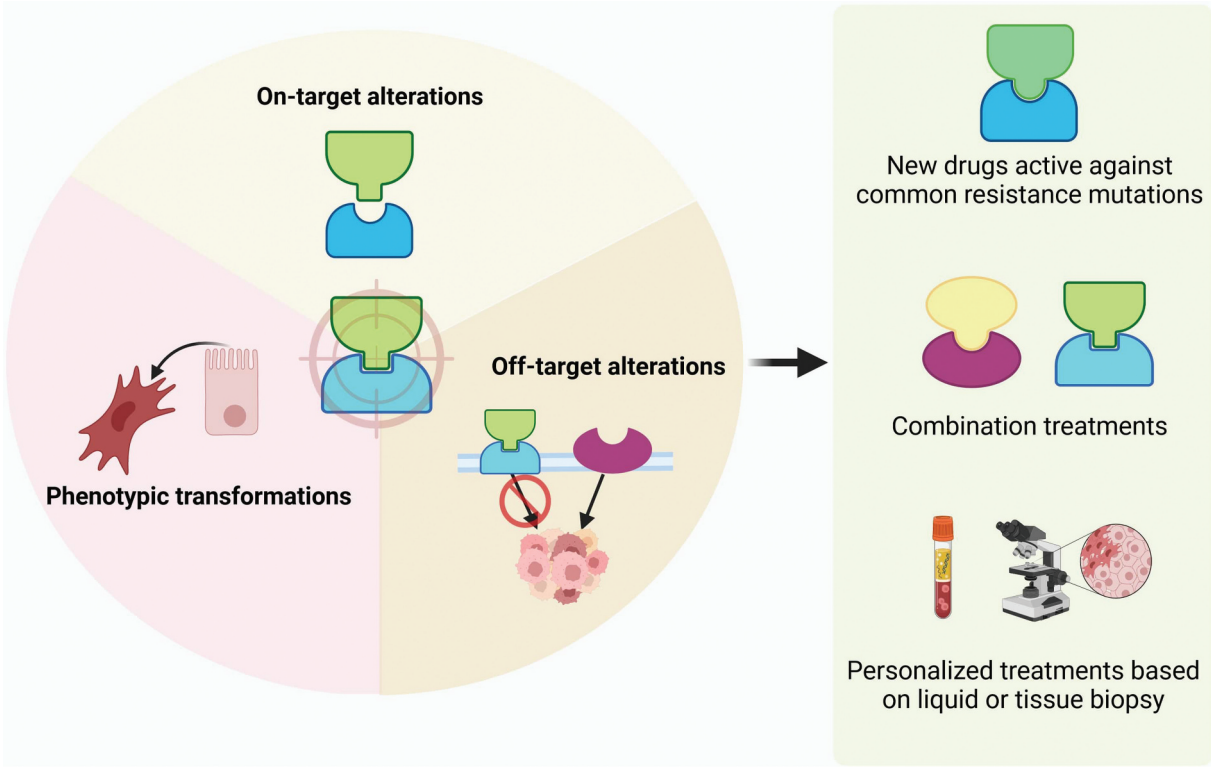

Figure 3. Mechanisms of resistance to target therapy and strategies to overcome them. there are three main mechanisms underlying resistance to targeted therapies: phenotypic transformations, on-target alterations and off-target alterations. Currently, attempts are being made to overcome this barrier by developing new drugs against common resistance mutations, combination of different treatments and personalized treatments based on liquid or tissue biopsy. Created with Biorender.com.

mortality rates, but modestly increased short-term survival rates without affecting long-term survival rates in PC patients [122].

Another important aspect of both PC and BTC is the TME and its role in favoring chemoresistance (Figures 1 and 2). A therapeutic option may involve reversing the protumorigenic signals of CAFs or transforming them into a non-CAFs phenotype 're-educating' CAFs to adopt a non-tumor-associated state [123,124]. One such approach involves the use of epigenetic modulating agents like the demethylating agent 5-Azacytidine (5-AZA). The administration of 5-AZA can transform an aggressive PC model into a less aggressive and more drug-sensitive phenotype. This transformation has been observed *in vivo*, with reduced tumor growth following engraftment of treated transformed cells. Additionally, when combined with GEM, 5-AZA exhibits a noticeable inhibitory effect on the growth of GEM-resistant PC cells [125]. A phase 2 trial with azacitidine and anti PD-1 pembrolizumab as second-line treatment in PC did not show improved PFS, however one patient had a notably long survival and maintained response, indicating the need for predictive biomarkers [126].

The stromal components in PC have been targeted using Hedgehog pathway inhibitors, such as IPI-926 or recombinant pegylated HA enzyme PEGPH20, however with unsatisfactory clinical outcomes [127,128]. A randomized phase-2 trial combining PEGPH20 with GEM and nab-paclitaxel in patients with high levels of HA only met the secondary endpoint of PFS and had to be temporarily halted due to increased thrombosis [128]. Similarly, a randomized phase II trial investigating front-line PEGPH20 combined with FOLFIRINOX in a non-biomarker-selected population was terminated early due to lack of effectiveness in the interim analysis [129]. Disappointing results have also been observed with inhibitors targeting the JAK2/STAT3 pathway, such as ruxolitinib, which did not show promising results in a phase-3 study when combined with capecitabine [130,131] (Table 3).

The first-line standard of care for more than 10 years before immunotherapy was included in the management of BTC was GEM with cisplatin, which was based on the groundbreaking phase III ABC-02 study that was published in 2010 [132]. The doublet regimen achieved a median progression-free survival (mPFS) of 8.0 months and median overall survival (mOS) of 11.7 months, a statistically significant improvement compared to 5.0 months and 8.1 months for GEM alone. Patients were randomized to receive either GEM/cisplatin or GEM monotherapy. The objective response rate (ORR) was 26.1% and the disease control rate (DCR) was 81.4% for the doublet arm.

Treatment alternatives for those without modifiable changes include 5-FU/LV with oxaliplatin (5-FU/LV plus FOLFOX) and 5-FU/LV plus nanoliposomal (nal-)irinotecan. FOLFOX produced an ORR of 4.9% in the phase III ABC-06 study, resulting in a substantial but clinically negligible improvement in OS of 6.2 months as opposed to 5.3 months with supportive treatment alone [16]. More recently, 5-FU/LV was examined in the second-line scenario with or without nal-irinotecan in the NIFTY phase IIb study. The revised extended follow-up data showed lesser differences, with the masked

ICR-assessed mPFS reported as 4.2 and 1.7 months, respectively, and the BICR-assessed mPFS of 7.1 months with nal-irinotecan vs. 1.4 months without nal-irinotecan [17].

A new stage II study assessing albumin-bound paclitaxel added to GEM-cisplatin in BTC detailed a medium overall survival of 19.2 months [133] in the meanwhile a stage III trial with this triplet versus the normal GEM-cisplatin doublet is not ongoing (NCT03768414) [134].

It is important to note that while these strategies show promise, overcoming chemoresistance remains a complex and ongoing research area. Clinical trials and translational studies are warranted to validate the effectiveness of these approaches in BTC and PCs.

3. Molecular mechanisms of resistance to target therapy

In the *era* of precision medicine, targeted therapies have emerged as a promising frontier in the treatment of various malignancies. These therapies, designed to specifically target aberrant molecular pathways driving cancer growth, have demonstrated remarkable efficacy in numerous tumors [135,136]. However, a formidable challenge persists: the development of resistance to these targeted interventions. As with conventional chemotherapies, cancer cells exhibit a remarkable capacity to adapt and evolve, rendering once-effective targeted therapies progressively less potent [137]. Broadly, there are three main mechanisms underlying resistance to target therapies [137] (Figure 3).

(1) On-target alterations: these include changes in the target receptor that cause the drugs to not be able to inhibit the pathway. A striking example of this mechanism can be found in EGFR mutated non-small cell lung cancer (NSCLC): the use of adenosine triphosphate (ATP)-competitive inhibitors such as gefitinib and erlotinib is often associated with the development of a T790M point mutation in the EGFR gene, causing an increase in affinity for the physiologic substrate ATP and thus decreasing the inhibition on the downstream pathway by the target drugs [138]. Other than single amino acid changes, on-target alteration can also include RNA alternative splicing, as has been seen in melanoma BRAF V600E patients treated with TKI, or gene amplification, as seen in imatinib-treated patients with chronic myeloid leukemia [139,140] (Figure 3).
(2) Off-target alterations: to bypass a drug's inhibition on a specific survival pathway, cancer cells often exhibit upregulation of different proliferation pathways. For example, BRAF V600E melanoma patients have to be treated with both BRAF and MEK inhibitors because the use of BRAF monotherapy leads to an upregulation in NRAS and a consequent mitogen-activated protein kinase (MAPK) reactivation [141]. Similarly, NSCLC treated with EGFR, anaplastic lymphoma kinase (ALK), RET, or reactive oxygen species (ROS)-1 inhibitors, often display a secondary MET amplification driving resistance [142] (Figure 3).
(3) Phenotypic transformation: histological changes have been described in several cancers treated with TKI [143]. EGFR mutated and ALK fusion-positive NSCLC treated with TKI can exhibit a transformation to small cell lung cancer, sometimes retaining the original mutation [144]. More commonly the phenotypic transformation takes the shape of an EMT, in which alterations in gene expression and transcriptional mechanisms give rise to cells with mesenchymal properties such as changes in cells' polarity, weakened cellular adhesion, increased migratory capacity, and cytoskeletal remodeling [145] (Figure 3).

To summarize, targeted therapies have managed to demonstrate remarkable efficacy by specifically attacking the aberrant molecular pathways that drive cancer growth. However, on-target, off-target and phenotypic changes can allow cancer cells to bypass the inhibition from target therapies. A comprehensive understanding of the underlying resistance mechanisms is essential to develop strategies to overcome them or to preemptively address them, thereby maximizing the long-term effectiveness of targeted therapies in the changing landscape of precision medicine.

3.1. Resistance to target therapy in biliary tract cancer

Although target therapies were added to the standard treatment paradigm of BTC only recently, the problem of treatment resistance has already emerged prominently. Due to a few clinical trials, research studies, and case reports focusing on different targets and drugs, we have garnered limited insight into the mechanisms that drive this phenomenon in BTC. On-target alterations have been detected using circulating tumor DNA (ctDNA) in liquid biopsy in patients treated with FGFR inhibitors. A small case series on eight patients with FGFR fusion or amplification reported that, at disease progression, 5/8 patients had developed mutations in the kinase domain of FGFR [146]. Interestingly each patient had up to 9 mutations, indicating a wide heterogeneity of resistance mechanisms. A similar pattern of development of multiple point mutations per patient was observed in the ctDNA of 2 out of 3 FGFR2 fusion-positive CCA patients progressing after treatment with infigratinib; furthermore, autopsy data on one of the patients showed that all 12 sampled metastases retained the FGFR fusion alteration but with different interlesional mutations, pointing to the idea that progression was not driven by selection of FGFR fusion-negative clones but from different convergent resistance mutations [147].

Regarding off-target alteration, it has been suggested that the co-presence of other mutations could be a cause for primary resistance to FGFR inhibitor, for example, 9 patients with both FGFR2 fusions and p53 mutations did not have an objective response to pemigatinib, and patients with CDKN2A or PBRM1 alteration had a shorter PFS [148]. Consistent with findings on colon cancer, primary resistance to EGFR therapy has also been noticed in CCA patients harboring a K-RAS mutation [149](Figure 1). Furthermore, off-target secondary resistance to FGFR inhibitors has been observed in both preclinical models, with a reactivation of MEK/ERK [150], and in the clinical setting, with the activation of the PI3K/AKT pathway [151].

In BTC there are complex interlaying mechanisms between the tumor microenvironment and it has been observed that cancer-associated fibroblasts can favor epithelial–mesenchymal transition, resistance to chemotherapy and cancer progression [152]. Furthermore, a preclinical paper suggests that epithelial–mesenchymal transition and acquisition of cancer stem cell properties can be a resistance mechanism in CCA cells treated with EGFR inhibitor erlotinib [153].

3.2. Resistance to target therapy in pancreatic cancer

The genetic landscape of PC has been well analyzed in search of targetable mutations, but monotherapy treatment with target drugs did not have much success in PC, possibly due to many dysregulated pathways with complex and multiple cross talks and the fact that the most commonly mutated genes do not have a corresponding target drug [58]. Thus, information on resistance mechanisms pertaining to PC specifically is scarce and limited to the few drugs that have been used in more advanced clinical trials.

For example, although KRAS alterations are present in around 90% of PC cases (Figure 2), only a very small subset exhibits the G12C alteration that makes it susceptible to the target inhibitor sotorasib [154]. However, even in these selected patients, the recorded ORR to second or further-line target therapy was only 21% [61]. Preclinical data point to the activation of alternative signaling pathways and the development of new mutations in the KRAS gene as possible secondary resistance mechanisms to KRAs inhibitor, ongoing trials are evaluating allele-specific inhibitors and pan-(K)RAS inhibitors against the more common allele variants G12D, G12V, and G12R 290 [155].

Furthermore, the presence of KRAS alteration has been linked to resistance to another type of target treatment, EGFR inhibitor. For example, the combination of anti-EGFR antibody nimotuzumab plus GEM had an OS advantage only in KRAS wild-type patients [156]. Many other trials with EGFR inhibitors (both TKI and monoclonal antibody) failed to prove a survival benefit in unselected pancreatic patients, again, probably due to alternative active pathways [157]. It should also be noted that, although the combination of erlotinib in association with GEM is associated with an OS gain [28], this regimen is not often used in clinical practice as the benefit in the trial was minimal.

One of the few target therapies that has been approved in PC is the PARP inhibitor, olaparib, in the setting of BRCA1/2 germline-mutated patients that had not progressed after platinum-based first-line therapy. This is quite a rare scenario in which two mechanisms of DNA repair are blocked, one related to the BRCA gene and one to PARP enzymes, making the cancer cells extremely susceptible to DNA damage mechanisms. Despite this encouraging premise, maintenance therapy with olaparib after platinum-based first-line therapy was not associated with a gain in OS (18.9 vs 18.1 months), but only a PFS gain (7.4 vs 3.8 months in the placebo arm) [27]. Although clinical data on PC patients are not available, resistance mechanisms to olaparib have been studied in pancreatic cell lines, pointing to the development of multiple genetic alterations including upregulation of MDR genes and phenotype changes such as EMT, furthermore, in other cancer, resistance to olaparib was also associated with 53BP1 downregulation and BRCA reversion mutations [158].

3.3. Overcoming target therapy resistance

Understanding the molecular mechanisms of resistance to targeted therapy is crucial for developing strategies to overcome or prevent resistance (Table 4). The first issue that needs to be addressed is how to recognize which resistance mechanisms are at play in each patient. Given the wide spectrum of resistance mechanisms and the high intra-tumoral heterogeneity of both BTC and PC, it is unlikely that a single lesion biopsy will unravel the full complexity of the resistance mechanisms that can arise in each patient. Thus, it is crucial to implement tools that will give a more comprehensive picture of the resistance mechanisms during cancer progression. In this context, the implementation of liquid biopsy is emerging as a very valuable tool.

A study on 23 patients with BTC reported an overall blood/tissue concordance at diagnosis of 74%, ranging from 55% per ECC and 92% for ICC [159], indicating the need for more in depth analysis on the applicability of liquid biopsy, especially in extrahepatic CCA. In PC, a meta-analysis on the use of liquid biopsy found an overall sensitivity of 70% and a specificity of 86% when compared to molecular analysis of tissue specimen; when looking at studies on *KRAS* mutations only, the sensitivity of liquid biopsy was a bit lower, around 65%, but with a high 91% specificity [160]. Of note, 78% of mutations detected in circulating free DNA were not found in the lesion biopsy, again confirming the likely inadequacy of single lesion biopsy [160].

Detecting the type or types of ongoing resistance mechanisms is only part of the problem, as then we need viable strategies to combat them (Table 4).

The development of target drugs that are active against the most common resistance mutations could be a noteworthy approach (Figure 3). For example, in EGFR-mutated NSCLC osimertinib, an irreversible TKI, active against a common resistance mutation associated with previous inhibitors, has led to significant increase in PFS [161]. In BTC this has been partially done with the development of futibatinib, an FGFR irreversible inhibitor active against some secondary kinase domain mutations arising from previous treatment with target therapy [162]. Furthermore, drugs such as erdafitinib are being tested not only in FGFR2 fusion positive cases but also in FGFR1–4 alteration, and another FGFR inhibitor, tinengotinib, has recently proved to be useful also in patients pretreated with FGFR inhibitors [163,164]. However, we still do not have a complete picture of which mutations can arise and will likely respond to each FGFR inhibitor on the market, so we are still away from a truly personalized approach.

Multiple new drugs are currently being tested. For example, the novel KRAS inhibitor (MRTX1133), targeting the G12D mutation, has already demonstrated preclinical efficacy, showing tumor regression in the majority of tested cell line xenografts (8 out 11) and patient-derived xenografts [165], and is now being tested in a clinical trials (NCT05737706) [166]. Another pan-KRAS inhibitor called BI-3406, currently in

Table 4. Overcoming target therapy resistance in BTC and PC.

Tumor type	Target	Regimen	Results	Authors	Year
ICC	EGFR (and FGFR)	Infigratinib + Futibatinib	– Tumor regression in vivo	Wu Q et al. [150]	2022
BTC	FGFR	TAS-120	– Notable inhibition in a huge spectrum of FGFR mutated cells	Goyal L et al. [162]	2019
PC	KRAS	MRTX1133	– Tumor regression in xenograft models	Wang X et al. [165]	2022
PC	KRAS	BI-3406	– Tumor growth suppression in xenograft models	Hofmann M. H et al. [167]	2021
PC	Fanconi Anemia pathway	AZD6738 + GEM	– Tumor growth inhibition in vitro – Anti-tumor efficacy in vivo – Drug sensitivity increased	Wallez Y et al. [172]	2018
PC	Fanconi Anemia pathway	VE-821	– Sensitization of normoxic and hypoxic PC cells to radiation/chemotherapy	Prevo R et al. [173]	2012
BTC	KRAS and mTORC1/2	Several compounds	– Inhibition of protein translation and cell survival pathways in vitro – Toxicity enhanced – Sustained and durable inhibition of growth of PDAC tumors in vivo – Prevention of metastatic formation in vivo – Cell death increased and resistance mitigated in vivo	Brown W. S et al. [175]	2020
PC	mTORC2 and PARP	PP242 + Olaparib	– Cell growth and invasion suppressed – Tumor cells more sensitive to drugs – Tumor size reduced in vivo	Bu C et al. [179]	2023
PC	Multi-targets	Nab-paclitaxel + GEM + Cisplatin + Irinotecan + Capecitabine + Pembrolizumab + Olaparib	– Study ongoing	NCT04753879 [185]	2023
PC	BRCA1/2 and PALB2	Niraparib + Dostarlimab	– Study ongoing	NCT04493060 [186]	2023

Abbreviations: ICC: intrahepatic cholangiocarcinoma, BTC: biliary tract cancer, PC: pancreatic cancer, EGFR: epidermal growth factor receptor, FGFR: fibroblast growth factor receptor, KRAS: kirsten rat sarcoma virus, PARP: poli-ADP ribose polymerase, GEM: gemcitabine, PC: pancreatic cancer.

phase-1 trials, disrupts the binding of KRAS to its activator SOS1 [167], and similarly another KRAS G12X inhibitor called RMC-6236 is being tested in at least two phase 1 trials (NCT05379985, NCT06128551) [168,169]. Additionally, the SHP2 inhibitor TNO155 has shown promising safety and tolerability, and multiple clinical trials are underway to test its efficacy in combination with other therapies [170,171].

Another emerging therapy for PC involves targeting the Fanconi Anemia (FA) pathway, which is involved in DNA cross-link repair: ATR inhibitors, which inhibit the downstream signaling of the FA pathway, have been shown to be effective in preclinical studies when combined with chemotherapy agents such as GEM [172,173] (Table 4).

A different strategy consists in combining multiple target drugs to block multiple pathways, similar to what has already been done in other cancers like melanoma [174] (Figure 3). Such combination therapies can help overcome and prevent resistance by blocking alternative signaling pathways or compensatory mechanisms. In preclinical BTC cell models derived from patients harboring FGFR2 fusion and that had progressed on FGFR inhibitor monotherapy, there was a synergic activity of EGFR and FGFR inhibitors [149]. Similarly, in a CCA cell line, a synergistic effect was observed with the use of a mTOR inhibitor plus a FGFR inhibitor [151]. This cell line was derived from a patient progressing to FGFR monotherapy due to upregulation of the PI3K/AKT/mTOR pathway, which is frequently upregulated in FGFR constitutionally active cancer cells and that can act as a bypass mechanism when FGFR is inhibited [151]. Again, this underlines the need to identify the mechanisms of resistance to allow for personalized treatments.

Similarly, in PC, the use of KRAS and MEK inhibitor has been associated with a compensatory activation of the AKT/mTOR pathway through cross talks proteins, thus the combination of either KRAS or MEK inhibitor along with an mTORC1/2 inhibitor has been used in preclinical models to prevent the activation of alternative pathways as a mechanism of resistance [175]. Several trials investigating combination therapies are underway, mostly in basket trials on solid tumors including PC, and involving different targets such as SHP2, ERK, MEK, KRAS, EGFR, VEGF, and CD4/6 [176–178] .Furthermore, given the use of olaparib in *BRCA1/2* mutated patients, one study explored the use of mTOR inhibitors in combination with PARP inhibitors, pointing to a synergic effect of this combination even in BRCA wild-type cell lines [179]. The combination of PARPi with immune checkpoint inhibitors has also been specifically used because BRCA insufficiency can trigger the production of type I interferon and pro-inflammatory cytokines, which can lead to an innate immune response that is dependent on the stimulator of interferon genes [180]. Furthermore, in a dose-dependent manner, clinical models have shown that PARP inhibition up-regulates PD-L1 and inactivates glycogen synthase kinase-3 (GSK3). As a result, T-cell activation is suppressed, which increases the death of cancer cells [181]. A phase 1/2 trial comparing PARPi niraparib plus nivolumab or ipilimumab as maintenance therapy after chemotherapy showed promising results in PFS for the niraparib–ipilimumab combination [182] and more trials are ongoing with olaparib plus pembrolizumab (NCT05093231, NCT04548752, NCT04753879) [183–185]

The combination of target therapy and immunotherapy is also being investigated (Figure 3). Currently, a phase-2 study (NCT04753879) is actively enrolling participants to evaluate the effectiveness of maintenance olaparib in combination with pembrolizumab after low-dose GEM, nab-paclitaxel,

capecitabine, cisplatin, and irinotecan in previously untreated metastatic PC patients [185]. Additionally, the National Cancer Institute is conducting a phase-2 trial (NCT04548752) comparing the use of olaparib alone versus the combination of olaparib and pembrolizumab in patients with metastatic germline BRCA1/2-mutated PC. Another trial (NCT04493060) is currently investigating the use of niraparib and dostarlimab, an anti-PD-1 antibody, in metastatic PC patients with BRCA1/2 and PALB2 mutations [186]. In BTC some ongoing trials include the anti IDH1 ivosidenib with nivolumab plus ipilimumab and the combination of PARP inhibitor olaparib plus durvalumab (NCT05921760, NCT03991832) [187,188]

In conclusion, resistance to targeted therapy is a complex phenomenon driven by various molecular mechanisms. By unraveling these mechanisms and developing innovative strategies, we can enhance the effectiveness of targeted therapies and improve patient outcomes.

4. Mechanisms of resistance to immunotherapy

Immunotherapy, heralded as a revolutionary paradigm in cancer treatment, has redefined the landscape of oncology by harnessing the intricate interplay between the immune system and malignant cells [189]. By empowering the body's immune defenses to recognize and combat cancer, immunotherapeutic agents have yielded unprecedented responses across a spectrum of malignancies. However, in PC, this class of drugs had to contend with high levels of primary resistance and the quick emergence of secondary resistance. The phenomenon of resistance to immunotherapy represents a complex interplay of biological mechanisms, whereby tumors employ a variety of strategies to evade or subvert the immune system's potent antitumor effects. Understanding the diverse molecular and cellular mechanisms that confer resistance to immunotherapy is paramount for advancing the effectiveness of these groundbreaking treatments. Broadly speaking there are two main components associated with immune evasion [190–192]. First, cancer cells possess intrinsic factors that play a crucial role in antigen presentation, a process vital for the immune system to recognize and eliminate cancer cells. However, loss of tumor neoantigen, changes in antigen presentation and processing, and hyperexpression of immune checkpoint proteins, can all help in the immune evasion. Furthermore, alteration of intracellular signaling due to genetic mutation, phenotypic transformation (such as EMT or a reversion to stem cell phenotype), and the secretion of metabolites to modify the tumor microenvironment are all possible causes of immune resistance as well. Second, the TME significantly shapes the presence, composition, and function of tumor infiltrating lymphocytes and other immune cells. The expression of immunosuppressive factors by the cancer cells, the presence of dysfunctional blood vessels, and a hostile, often hypoxic, environment are some of the factors contributing to immune evasion.

By illuminating the dynamic adaptations that underlie resistance, we aim to catalyze the development of strategies capable of overcoming these barriers and extending the transformative potential of immunotherapeutic approaches in the fight against BTC and PC.

4.1. Resistance to immunotherapy in BTC

Immunotherapy has been proven to prolong survival in BTC and has now become part of the first-line treatment, in combination with cisplatin and GEM. However, the number of patients responding to treatment is less than 30%, pointing to high levels of primary resistance to treatment (Figure 1), and biomarkers of response that have been used in other cancers, such as PD-L1, have not been useful in BTC [5,7].

As BTCs are known to be very heterogeneous, several attempts have been made to classify them and possibly predict a response to therapy. For example, a genomic analysis divided BTC into 4 different categories, and it points out the presence of a cluster (cluster 4) as having both a high mutational load, causing elevated levels of neoantigens, and also having a high expression of immune checkpoint genes involved in suppressing an immune response [193]. Furthermore, a paper on TME in iCCA points out the presence of four subtypes: the I1 immune-desert subtype is associated with feeble tumoral and stromal immune signaling, the I2 immunogenic subtype has a strong lymphoid and myeloid response, I3 myeloid subtypes displays only a strong myeloid response and the I4 mesenchymal subtypes is associated with activated fibroblasts, EMT, stem cells like features and neoangiogenesis[174]. This classification reflects survival rates with the I4 subtype having shorter survivals and I2 subtypes having better survival rates [194]. We can then speculate that different subtypes could respond differently to immunotherapy, but no correlation between clinical data and response to different therapies is available.

Subclassification aside, several papers point to an intrinsic ability of BTC cells to suppress an immune response (Figure 4). The secretion of PDGF-D and TGF-β by cancer cells causes the activation of CAFs, which in turn promotes the creation of a pro-inflammatory response with low CD8+ T cells and antigen presenting cells, and high Tregs and myeloid-derived suppressor cells [195]. Furthermore, around half of BTC were found to have a low antigen presenting molecules (MCH)-1 expression, associated with worse survival rates and the levels of MCH-1 were also correlated with tumor associated lymphocytes and macrophages [196] (Figure 4).

It should also be noted that the presence of certain mutations could influence immune response. For example, IDH1 mutations cause an accumulation of the oncometabolite D-2-hydroxyglutarate, and, at least in gliomas, is associated with low CD8+ T cells and repression of the tumor immune system [197]. Furthermore, FGFR signaling has been associated with a decrease in MCH-II, upregulation of PD-L1 and increase of regulatory T cells, again leading to an immune suppressive environment [198].

To conclude, the causes of immune evasion in BTC are numerous (Figure 4); however, data on how these mechanisms relate to response to immunotherapy is lacking.

4.2. Resistance to immunotherapy in pancreatic cancer

Immunotherapy is not effective for PC, despite the increased knowledge of the genomic landscape and of the complex TME (Figure 5). Although it can be considered in patients with MSI or high tumor mutational burden, in this small subset of

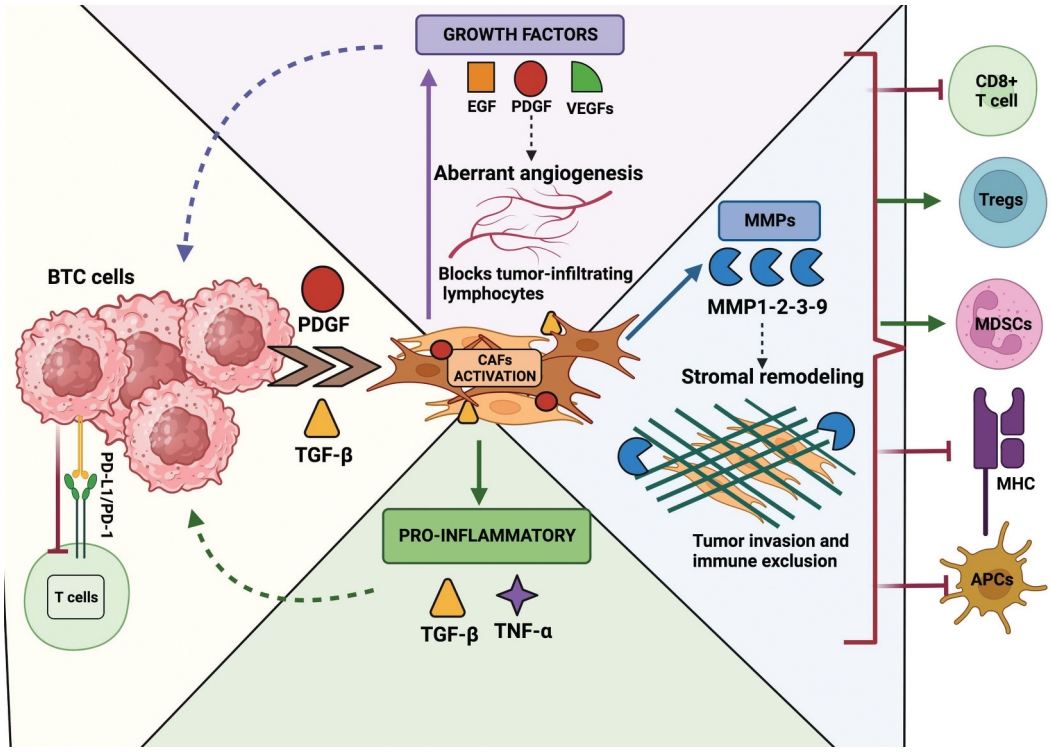

Figure 4. Immunoresistance mechanism in BTC. tumor cells secrete PDGF and TGF-β which helps to recruit a large amount of TME CAFs through PDGFR-β signaling, these activated CAFs can release (1) proinflammatory compounds such as TGF-β and TNF-α that reinforce production by part of the tumor cells themselves; (2) ECM remodeling factors including MMP1, MMP2, MMP3, and MMP9; (3) growth factors including EGF, PDGF-B, and angiogenic factors, such as VEGF. VEGF isoforms, in turn, activate multiple VEGFRs (VEGFR1–3) that are found in a wide variety of endothelial and lymphatic cells, immune cells, and tumor cells themselves. Created with Biorender.com.

patients (around 2%), ORR to pembrolizumab has been reported between 62% and 18%, likely thanks to the high number of neoantigens being produced, capable of stimulating the immune system [31,199].

One culprit of immunotherapy's discouraging results is certainly the TME: PCs have a dense and prominent stroma, rich with immunosuppressive cells such as regulatory T-cells (Treg), suppressive myeloid cells [200], CAFs and TAMs, but lack of effector T cells [201](Figure 5). However, different compositions of TME have been described, with more mature-intermediate-immature characteristics, that correlate with prognosis, so it is possible that different subtypes may have different responses to immunotherapy [202]. Furthermore, PC cells often exhibit low expression of tumor antigens, limiting the ability of the immune system to mount an effective response, and they can upregulate immunosuppressive proteins such as PD-L1 and Cytotoxic T-Lymphocyte Antigen 4 [202] (Figure 5).

4.3. Overcoming immunotherapy resistance

Overcoming immunotherapy resistance in cancer is a major focus of ongoing research and clinical trials and several strategies are being explored to enhance the effectiveness of immunotherapy.

First of all, a better understanding of which patients will benefit from immunotherapy will certainly be helpful, but unfortunately, markers used in other cancers, such as PD-L1, did not prove to be useful. For example, the use of pembrolizumab in pretreated BTC patients was associated with ORR between 6% and 13% with no discernible association with PD-L1 expression [203]. On the other hand, the use of nivolumab in the same subset of patients saw similar ORR results (11%) and a significant correlation between PFS and PD-L1 expression, however, most notably, all responders to treatment had MSI [204]. Currently, both in BTC and PC, the use of immunotherapy alone is only recommended in MSI cases, however, there could be other subsets of patients that could benefit from it. For example, a small case series on 12 BTC or PC patients with homologous recombination deficiency showed that the use of combination immunotherapy with nivolumab and ipilimumab was associated with an ORR of 42%, with 4 patients reaching a complete response. Furthermore, the responders had a much higher level of TIL compared to non-responders, with a T cell inflamed signature on RNA expression analysis [205]. Furthermore, as stated before, several papers have tried to subclassify BTC and PC according to the different levels of immune suppression, but so far these classifications were not correlated to response to immunotherapy [193,194,202] (Table 5).

Other than a better selection of patients, equally important is the development of better and more effective approaches to stimulate the immune system and combat immune resistance.

Over the years, a viable strategy has been the use of combination therapies (Figure 3). The combination of different drugs that can target multiple immune checkpoints has had conflicting results: for example, the use of anti PD-L1 and anti CTLA4 was associated with ORR of around 20% when involving nivolumab and ipilimumab [206], while with the combination of

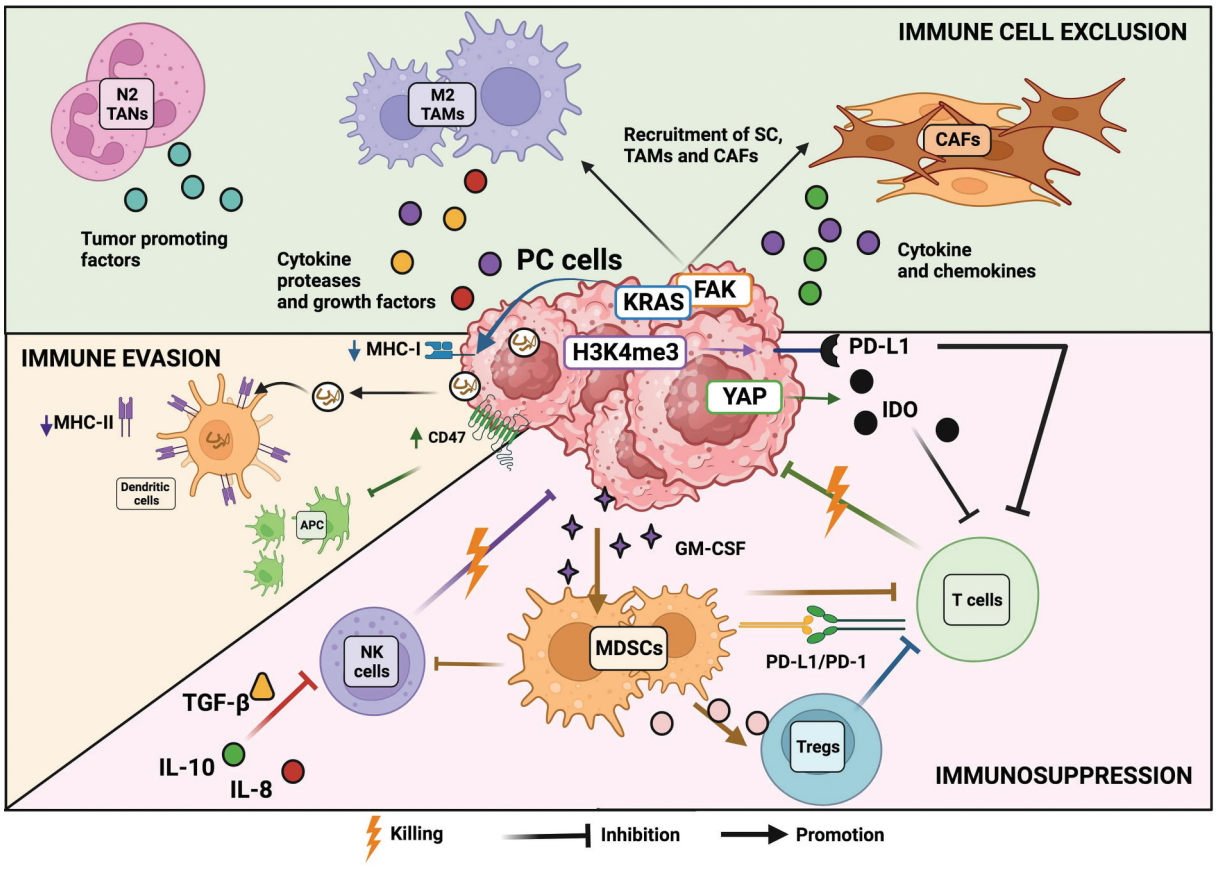

Figure 5. Immunoresistance mechanism in PC. (a) The tumor cells recruit and malignant the fibroblasts and macrophages of the tumor environment, thus the extracellular matrix becomes highly fibrotic and creates a physical barrier that prevents the infiltration of T cells into the tumor. (b) Immune evasion tactics in pancreatic cancer include: KRAS produced by the tumor cell reduces the expression of MHC I; just as miRNA-containing exosomes from tumor cells silence the expression of MHC II molecules in dendritic cells; and increased expression of antiphagocytic molecules such as CD47 prevents APC processing and tumor clearance. (c) The tumor suppresses immune responses through the expression of PD-L1, the recruitment of MDSC that activates regulatory T cells and inhibits T cells and NK cells, as well as the high expression of IDO that also allows them to block to the T cells. Created with Biorender.com.

durvalumab and tremelimumab the ORR was less than 5% [207]. Moreover, other combinations are being tested with drugs working on different mechanisms of action, such as immunotherapy plus targeted therapy or chemotherapy. As stated before, the combination of chemo-immunotherapy is now the standard first-line in BTC, but many more are being evaluated such as anti VEGFR regorafenib + avelumab, or pembrolizumab + Lenvatinib, or chemotherapy plus lenvatinib and anti-PD-1 toripalimab [208–210]. Furthermore, given the immunosuppressive nature of some genetic alterations, the idea of combining target inhibitor and immunotherapy is being explored: anti IDH1 Ivosidenib + nivolumab, anti FGFR2

Table 5. Overcoming immunoresistance in BTC and PC.

Tumor type	Regimen	Phase	Number of patients enrolled	Median OS (months)	Authors	Year
PC and BTC	Ipilimumab + Nivolumab	N.S	12	N.S	Terrero G et al. [205]	2022
PC	Atezolizumab + Autogene Cevumeran + mFOLFIRINOX	I	34	18	Rojas L. A et al. [216]	2023
BTC	Pembrolizumab	II	24 and 104	5.7	Piha-Paul S.A et al. [203]	2020
BTC	Nivolumab	II	54	14.2	Kim R. D et al. [204]	2020
BTC	Ipilimumab + Nivolumab	II	39	5.7	Klein O et al. [206]	2019
BTC	Durvalumab + Tremelimumab	II	116	10.1	Doki Y et al. [207]	2022
BTC	GEM/Cisplatin + Durvalumab	III	128	12.8	Oh D. Y et al. [5]	2022
BTC	GEM/Cisplatin + Pembrolizumab	III	1564	12.7	Kelley R. K et al. [7]	2023
BTC	Lenvatinib + Pembrolizumab	II	32	11.0	Lin J et al. [208]	2020
BTC	Regorafenib + Avelumab	II	34	11.9	Cousin S et al. [209]	2022
BTC	Gem/Oxiplatin + Lenvatinib + Toripalimab	II	30	22.5	Shi G.-M et al. [210]	2023
BTC	EGFR CAR-T	I	19	-	Guo Y et al. [211]	2018
BTC	Allogeneic NK cells + Pembrolizumab	I/II	40	-	Leem G et al. [218]	2022
PC	IMP321 + GEM	I	18	25	Wang-Gillam A et al. [213]	2013
PC	GEM + Nab-paclitaxel + Indoximod	II	135	10.9	Bahary N et al. [214]	2018

Abbreviations: N: number of patients, BTC: biliary tract cancer, PC: pancreatic cancer, N.S: not said, OS: overall survival, GEM: gemcitabine, mFOLFIRINOX: oxaliplatin, leucovorin, irinotecan and fluorouracil, NK: natural killer, EGFR: epidermal growth factor receptor.

futibatinib + pembrolizumab, anti MEK cobimetinib + atezolizumab, or the implementation of chimeric antigen receptor-engineered autologous T (CAR-T) cell immunotherapy directed against EGFR positive cells, just cite a few [195,211] (Table 5). Furthermore, other than PD-L1 or CTLA4 inhibition, novel immune targets are being explored, alone or in combination with other immune-stimulating drugs, with the intent to modify the immunosuppressive effect of the tumor microenvironment and boost the effect of immune cells. Some of these targets are, for example: TIM-3, LAG-3, TIGIT, and VISTA plus in PC specifically CD40, CD11b, OX40, IDO, and B7/H3 [212–215].

Many other innovative immune strategies are in the early stages of testing. For example, the use of vaccines or oncolytic viruses, to enhance antigen presentation and improve the recognition of tumor cells by the immune system [216]. Furthermore, the use of adoptive cell transfer therapy is being explored in different types of solid tumors, including BTC and PC [217]. It involves genetically modifying a patient's own immune cells, such as T cells or natural killer cells, to express chimeric antigen receptors or T cell receptors that specifically recognize tumor antigens; These modified cells are then infused back into the patient to target and kill cancer cells. For example, an early trial with the combination of allogeneic natural killer cells in combination with pembrolizumab showed good tolerability and some antitumor activity [218]. Finally, since epigenetic modifications can influence the expression of genes involved in immune response regulation, the modulation of these epigenetic changes through specific drugs has the potential to sensitize the tumor to immunotherapy and overcome resistance: for this reason, several new drugs such as DNA methyltransferase inhibitors, HDAi, BET inhibitors, and EZH2 inhibitors are being tested [219] (Table 5).

Furthermore, several phase II studies are currently underway investigating the possibility of incorporating immunotherapy into the adjuvant treatment of BTC, as It is possible that an early use of immunotherapy could lead to less treatment resistance. Combinations being explored include capecitabine plus lenvatinib and tislelizumab (NCT05254847 [220]), capecitabine plus camrelizumab and radiotherapy (NCT04333927 [221]), and capecitabine with durvalumab and tremelimumab (NCT05239169 [222]). Furthermore, a phase II trial (NCT04506281 [223]) is ongoing using the combination of oripalimab plus gemcitabine-oxaliplatin plus lenvatinib, which was previously studied in advanced/metastatic cases as a treatment for resectable iCCA, with promising results [210].

To conclude, the road to overcome resistance to immunotherapy is still long. The lack of predictive tools to implement a better selection of patients that will likely benefit from immunotherapy and the complex immunosuppressive environment of both BTC and PC, are significant challenges that need to be overcome. Hopefully, an even better understanding of the resistance mechanisms at play and the ever-changing new ideas and technologies being studied will allow for a breakthrough soon.

5. Conclusions

Treatment resistance in pancreatic and biliary tract cancer is a significant challenge in the field of oncology, as it concerns all available therapies, from chemotherapy, to target therapy and immunotherapy. As these cancers have limited treatment options, shedding light on these hidden mechanisms of treatment resistance can pave the way for groundbreaking advancements in the fight against cancer.

The molecular mechanisms of resistance are numerous, complex, and often intertwined, as they include processes that are inherently connected: genetic alterations, altered drug metabolism and efflux, changes in the tumor microenvironment, and phenotypic transformations (Figures 1 and 2). Unraveling the complexities of these mechanisms is essential to develop strategies to overcome or prevent resistance, ushering in a new era of personalized medicine, where tailored treatments can effectively combat chemoresistant cancers.

Despite the existence of multiple current therapies that are being employed to combat different levels of chemoresistance (Tables 3–5), it is imperative to emphasize the role of collaborative interdisciplinary research in the pursuit of overcoming treatment resistance in PC and BTC. Integrating insights from diverse scientific disciplines, including genomics, pharmacology, immunology, and computational biology, can provide a more comprehensive understanding of the intricate network of factors contributing to resistance. Furthermore, advancements in diagnostic tools and technologies, such as liquid biopsies and high-throughput sequencing, hold promise in identifying early markers of treatment resistance. These tools not only enhance our ability to monitor the dynamic changes in the molecular landscape of tumors but also enable timely adjustments to therapeutic interventions.

Combination therapies, modulation of the tumor microenvironment, new drugs and personalized medicine approaches are being explored as potential solutions (Figure 3). To confirm the efficacy of these strategies, particularly in complex and heterogeneous cancers like BTC and PC, additional studies and clinical trials are required.

In conclusion, the fight against treatment resistance in PC and BTC necessitates a multi-faceted approach that encompasses not only therapeutic strategies but also collaborative research endeavors and the integration of cutting-edge technologies. By embracing the complexity of these challenges and leveraging the collective expertise of the scientific community, we can pave the way for transformative breakthroughs in the battle against chemoresistant cancers.

6. Expert opinion

Considering previous studies on drug resistance in pancreatic and biliary tract cancer, the scientific community is still far away from having a concise picture of how cancer cells are able to survive the treatments that are routinely administered to patients.

In this review, we provide a concise picture of the mechanisms that have been discovered so far, in order to underline the astounding number of mechanisms that have been observed and studied in these cancers. What is truly lacking then is a solid theory of how all these different mechanisms combine to make both pancreatic and biliary tract cancers so challenging to treat. Unfortunately, the development of

a unified theory on resistance to therapy is made even more challenging by the high heterogeneity of these cancers, so that the main mechanisms of resistance can be different not only between patients but also within different metastatic lesions of the same patients, or even different tumor areas of a single nodule [224,225].

Given how complicated the topic is, perhaps a different approach needs to be taken to the issue. One suggestion is to address each patient as an individual rather than concentrating on PC or BTC in general. This translates into the notion of creating a tool that can assist in identifying the primary mechanisms at work to better target the cancer in practice. This might take the form of a liquid biopsy, but it might also be a deeper single-cell transcriptome study of the tissue sample or perhaps another as-yet-undeveloped technology [160,226]. Indeed, an article on identifying resistance mechanisms in gastrointestinal cancer points to a better clinical relevance of the results obtained with liquid biopsy compared to tissue biopsy, as a liquid biopsy gave a more complete picture of the various ongoing resistance mechanisms while tissue biopsy could only identify the mechanisms relevant to the small acquired tissue sample [227]. Furthermore, to better probe intra-tumoral heterogeneity, analysis of transcriptomic data from single cells are being performed to predict drug sensitivity and test selective drugs that could overcome treatment resistance, although these types of tests are only recently starting to be implemented in the clinical setting [228,229].

Given the rapid advances in computer technology, another emerging possibility is the use of artificial intelligence (AI) to integrate all the data available on different treatment resistance mechanisms. The use of AI could enable us to truly have a unified picture of the mechanisms of resistance to therapy, and consequently, it could help point scientists in the right direction for the development of more useful strategies to overcome resistance [230–232]. AI has already been used to identify genes that are likely to be involved in modifications related to drug resistance, including epigenetic changes, and to predict which drugs are more likely to advance from the preclinical to the clinical setting, helping reduce the costs and time spent on dead-end projects [230,231]. In the setting of PC, advances in AI have already been made regarding early diagnosis, both with biomarkers and radiological imaging, while in the early stages of both PC and BTC, AI is being used to aid surgeons in cases of uncertain diagnostic imaging or for planning of complex surgery [233,234]. Hopefully, the implementation of AI in both PC and BTC to better understand the mechanisms of drug resistance will be part of the near future.

A more in-depth knowledge of the resistance mechanisms is helpful to guide treatment decisions, but it is only useful when combined with the development of new drugs or improvement of drugs that are already available. Small steps in this direction have already been made, for example, the development of new generation FGFR inhibitors in BTC, active against some resistance mutation to previous drugs, or the use of maintenance therapy with PARP inhibitor olaparib in BRCA1/2 mutated PC patients [235]. Although these therapies are only applicable to a small subset of patients, we believe that they should be seen as a success and as herald drugs in the new landscape of personalized medicine. Hopefully, this type of personalized treatment can continue to be explored, even though the challenges are not only scientific in nature but also economical, as the road to developing drugs that can only be used in a small subset of patients is often arduous.

To conclude, the current therapeutic options for PC and BTC, including chemotherapy, targeted therapy, and immunotherapy, have shown limited success in overcoming treatment resistance. Our expert opinion is that a deeper understanding of each patient's resistance mechanisms could really help in implementing the right drugs to prolong survival. To make these tailored treatments possible, it is also paramount that scientists keep working on novel approaches. We believe that an improvement in treatment results for these aggressive malignancies is feasible through a deeper comprehension of the molecular causes of resistance, the creation of customized methods, and the investigation of innovative combination medicines.

Abbreviations

5-AZA	5-Azacytidine
ABC	ATP-binding Cassette
ACT	Adoptive Cell Transfer
AI	Artificial Intelligence
AKT	Serine/Threonine Kinase
ALK	Anaplastic Lymphoma Kinase
ATM	Ataxia Telangiectasia Mutated
ATP	Adenosine Triphosphate
Bcl-2	B-Cell Lymphoma 2
BRAF	B-Raf Proto-oncogene, Serine/Threonine Kinase
BTC	Biliary Tract Cancer
CAFs	Cancer-associated Fibroblasts
CCA	Cholangiocarcinoma
ctDNA	Circulating Tumor DNA
DCR	Disease Control Rate
DNA	Deoxyribonucleic Acid
DSB	Double Strand Breaks
ECC	Extrahepatic Cholangiocarcinoma
ECM	Extracellular Matrix
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial-mesenchymal Transition
ERK	Extracellular Regulated Kinase
FA	Fanconi Anemia
FDA	Food and Drug Administration
FGFR	Fibroblast Growth Factor Receptor
GEM	Gemcitabine
HA	Hyaluronic Acid
hENT	Human Equilibrative Nucleoside Transporter
HDR	Homology-directed Repair
ICC	Intrahepatic Cholangiocarcinoma
IDH1	Isocitrate Dehydrogenase 1
KRAS	Kirsten Rat Sarcoma Virus
MAPK	Mitogen-activated Protein Kinase
MCH	Antigen Presenting Molecules
MSI	Microsatellite Instability
NF-kβ	Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells
NSCLC	Non-small Cell Lung Cancer
NTRK	Neurotrophic Tyrosine Receptor Kinase
ORR	Overall Response Rate
OS	Overall Survival
PARP	Poly (ADP-ribose) Polymerase
PARPi	Poly (ADP-ribose) Polymerase Inhibitor
PC	Pancreatic Cancer
PD-L1	Programmed Death-ligand 1
PDX	Patient-Derived Xenograft

PFS	Progression Free Survival
PI3K	Phosphoinositide-3-kinase
PLOD2	2-Oxoglutarate 5-Dioxygenase 2
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
Shh	Sonic hedgehog
TAM	Tumor Associated Macrophage
TGF-β	Transforming Growth Factor Beta
TKI	Tyrosine Kinase Inhibitor
TME	Tumor Microenvironment
Treg	Regulatory T-cells
VEGF	Vascular Endothelial Growth Factor
ZEB1	Zinc Finger E-box Binding Homeobox 1

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Declaration of interest

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Author contributions

B Toledo and C Deiana wrote the manuscript. E Giovannetti revised and corrected thoroughly the manuscript. All authors revised the manuscript critically and agreed to the published version of the manuscript.

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