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Evidence Review

EVIDENCE REVIEW

Refining the Treatment of Pancreatic Cancer From Big Data to Improved Individual Survival

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Abstract

Pancreatic cancer is one of the most lethal cancers worldwide, most notably in Europe and North America. Great strides have been made in combining the most effective conventional therapies to improve survival at least in the short and medium term. The start of treatment can only be made once a diagnosis is made, which at this point, the tumor volume is already very high in the primary cancer and systemically. If caught at the earliest opportunity (in circa 20% patients) surgical resection of the primary followed by combination chemotherapy can achieve 5-year overall survival rates of 30%–50%. A delay in detection of even a few months after symptom onset will result in the tumor having only borderline resectabilty (in 20%–30% of patients), in which case the best survival is achieved by using short-course chemotherapy before tumor resection as well as adjuvant chemotherapy. Once metastases become visible (in 40%–60% of patients), cure is not possible, palliative cytotoxics only being able to prolong life by few months. Even in apparently successful therapy in resected and borderline resectable patients, the recurrence rate is very high. Considerable efforts to understand the nature of pancreatic cancer through large-scale genomics, transcriptomics, and digital profiling, combined with functional preclinical models, using genetically engineered mouse models and patient derived organoids, have identified the critical role of the tumor microenvironment in determining the nature of chemo- and immuno-resistance. This functional understanding has powered fresh and exciting approaches for the treatment of this cancer.

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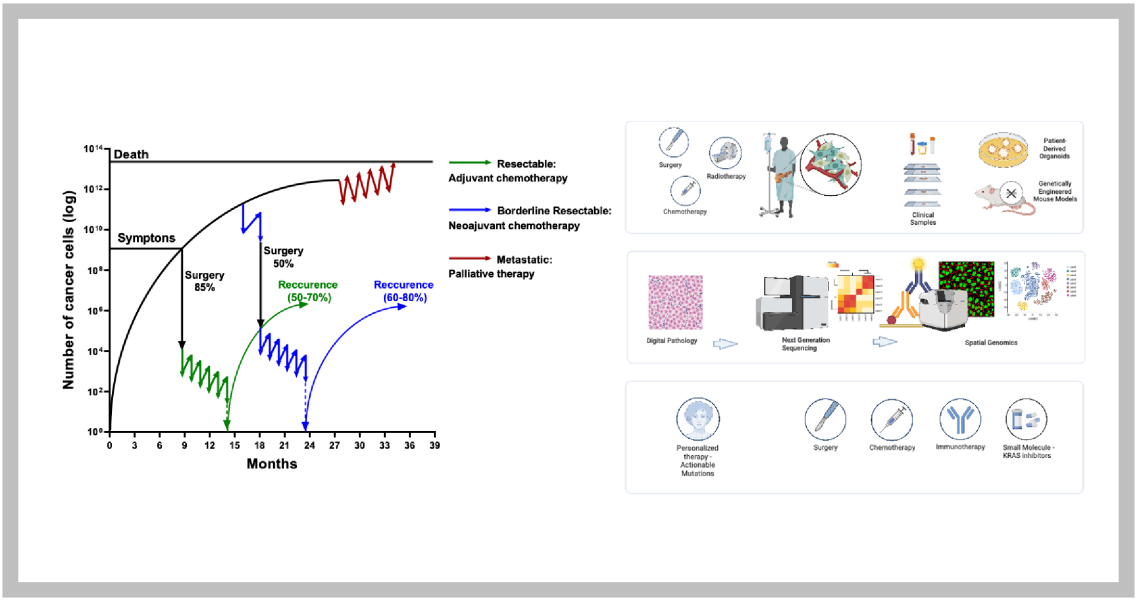

Key words: molecular subtypes; clonal evolution; plasticity; CYPY3A; targeted therapies; immunotherapy; persister

Introduction

Pancreatic cancer—pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with a mortality ranking in 2020 of seven worldwide, six in Northern Europe and four in the United States.[1,2] From the outset, it is an aggressive tumor becoming invasive and metastatic within 11–13 months from detection and highly resistant to all forms of treatment.[3,4] The mechanisms underlying this biological phenotype are not sufficiently understood but are being intensively investigated from many angles by different specialist teams around the world. In 2008, in depth sequencing of 24 PDAC tumors revealed an average of 63 genetic alterations, the majority of which are point mutations.[5] These alterations defined a core set of 12 cellular signaling pathways and processes that were each genetically altered in 67 to 100% of the tumors. These 12 pathways (with the fraction of tumors with genetic alteration of at least one of the genes) comprised KRAS signaling (100%), DNA damage control (83%), regulation of G1/S phase transition (100%), TGFβ signaling (100%), apoptosis (100%), Hedgehog signaling (100%), homophilic cell adhesion (79%), integrin signaling, c-Jun N-terminal kinase signaling (96%), regulation of invasion (92%), small GTPase-dependent signaling (79%), and Wnt/Notch signaling (100%).[5] To these, we can now also add genetic alterations in histone modulation (25%), SWI/SNF ATP-dependent chromatin remodeling complexes (20%), RNA processing (15%), and the ROBO/SLIT pathway (5%) (Table 1).[6–9] The most common single gene alterations occur in KRAS (90%), TP53 (70%), CDKN2A (60%), and SMAD4 (40%) whilst TGFBR2, ARID1A (SWI/SNF subunit), KDM6A (histone demethylase), MLL3 (histone H3K4 methyltransferase), RBM10 (RNA-binding motif-10 regulating alternative splicing), BCORL1 (transcriptional corepressor), and ROBO2 (roundabout guidance receptor 2, limiting stromal T-cell infiltration) occur in 5%–10% of tumors.[10,11]

The average number of genetic alterations in PDAC tumors is insufficient to explain the extremely poor prognosis and response to therapy, since other tumors with a much better prognosis have a larger number of average mutations notably breast and colorectal cancers whilst the genetic spectra of colorectal, brain, and pancreatic tumors are similar.[5]

To meet the challenge of a better understanding of how to effectively treat pancreatic cancer, there has been a huge investment in genomic and transcriptomic data acquisition.[12–14] So far this has led to only marginal therapeutic advances, since the clinical context of this data collection has often been lacking.[11,14] This paucity of progress was highlighted by Paul Nurse referring to Sydney Brenner, on receiving his Nobel Prize that we are "drowning in a sea of data and starving for knowledge." Ole Petersen has again referred to this dilemma urging that to "re-establish a focus on what really matters, namely, to gain useable knowledge from data, it would seem a good idea to rethink our working and assessment culture and start by placing much more emphasis on theory and model building as well as context."[15] A multidisciplinary approach is required to build validatable models that must combine both empirical and reductionist approaches.[16]

Opportunities and Limitations of Current Therapies

Cytotoxic Therapies—The Empirical Drive

The treatment of pancreatic cancer has evolved into different therapies based upon the cancer stage of disease. Traditional staging relies on pathological staging using the tumor, lymph node and metastasis (TNM) system, typified by the Union for International Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC) classifications.[17,18] Given the central role of surgical resection in the overall management of pancreatic cancer, an empirical system has emerged comprising five stages: resectable, borderline resectable, locally unresectable, oligometastatic disease, and large volume metastatic disease.[16,19,20]

There have been several 1000 clinical trials conducted in pancreatic cancer over the past 50 years. Most of these stud-

Table 1. Pathogenic gene variants occurring in 5% or more pancreatic cancers

Cellular Signaling Pathways and Processes	Pathogenic Gene Variants
KRAS signaling	KRAS, MAP2K4, RASGRP3
DNA damage control	TP53, ERCC4, ERCC6, EP300, RANBP2, BRCA1/2, PALB2, ATM, ATR, MLH1, MSH2, MSH6, RPA1, STK11, FANCA, FANCC, ATF2
Regulation of G1/S phase transition	CDKN2A, CHD1, APC2, FBXW7,
TGFβ signaling	SMAD4, SMAD3, TGFBR1, TGFBR2, BMPR2, ACVR1B, ACVR2A
Apoptosis	CASP10, VCP, CAD, HIP1
Hedgehog signaling	TBX5, SOX3, LRP2, GLI1, GLI3, BMPR2, CREBBP
Homophilic cell adhesion	CDH1, FAT, PCDH15, PCDHB16, PCDHGA1
Integrin signaling	ITGA4, LAMA1, LAMA4, LAMA5 FN1, ILK,
c-Jun N-terminal kinase signaling	MAP4K3, TNF, ATF2, NFATC3
Regulation of invasion	ADAM11, DPP6, MEP1A, PCSK6, APG4A
Small GTPase-dependent signaling	AGHGEF7, ARHGEF9, CDC42BPA
Wnt/Notch signaling	JAG1, BCORL1, NF2, FBXW7, RNF43, MARK2, TLE4, MYC, PPP2R3A, WNT9A, MAP2, TSC2, GATA6.
Histone modulation	KDM6A, MLL2, MLL3, SETD2
SWI/SNF ATP-dependent chromatin remodeling	ARID1A, ASD1B, PBRM1, SMARCA4
RNA processing	RNMI0, SF3B1, U2AF1
ROBO/SLIT pathway	ROBO1, ROBO2, SLIT2, MYCBP2

Genes underlined are most altered in pancreatic cancers.

ies have been phase I/II studies assessing the potential survival benefit (with at least manageable toxicity) of newer agents and other treatment modalities such as chemoradiation, but unfortunately most have met with failure. Today, there are over 3000 PDAC trials registered at clinicaltrials.gov.in with 1099 studies currently in phase I, 271 in phase I/II, 1441 in phase II, and 306 studies in phase III, including 254 neoadjuvant trials of which 23 are in phase III. The EU Clinical Trials Register presently records 487 PDAC trials, phase III in 87, of which 10 are neoadjuvant trials. In unravelling this huge amount of information, it is important to focus on well-conducted phase III randomized trials with appropriate control arms. The mainstay of systemic treatment is combination chemotherapy, and it is perhaps surprising how relatively few agents and combinations show sufficient efficacy to meet with regulatory approval or become standard-of-care[4,21–29] (Table 2). In metastatic disease compared to gemcitabine monotherapy with a median overall rate of 5.7 months,[22] combination treatments are a distinct advantage with median overall survival rates of 7.1 months for gemcitabine + capecitabine (GEM-CAP),[30] 8.7 months for gemcitabine + nab-paclitaxel (GnP),[25] 11.1 months for folinic acid + 5-fluorouracil (5FU) + irinotecan + oxaliplatin (FOLFIRINOX);[24] and the combination of liposomal irinotecan + 5FU/leucovorin and oxaliplatin (NALIRIFOX) is also superior to GnP.[29]

The best results are achieved in patients with resectable tumors followed by (adjuvant) chemotherapy.[16,27,28,31–36] In the ESPAC-4 study with broad inclusion criteria, the addition of capecitabine to gemcitabine increased 5-year overall survival from 16.3% to 28.8%,[27] whilst FOLFIRINOX in the PRODIGE24 study improved the 5-year overall survival to 43.2% compared to 31.4% with gemcitabine monotherapy, although with more selective criteria in patients <79 years.[28,37]

In patients with borderline resectable disease, the administration of chemotherapy prior to resection (neoadjuvant therapy) in addition to adjuvant therapy achieves superior survival compared to adjuvant therapy alone.[38,39] In radiologically unresectable disease, a long course of combination chemotherapy can result in resectabilty rates of up to 60% in selected patients with a substantial improvement in median and 5-year survival rates compared to patients in whom the tumor cannot be removed.[40,41] Better survival rates are also being reported in selected patients with oligometastatic disease to the lung and liver.[16,42,43] The role of chemoradiation for survival improvement in pancreatic cancer is controversial, as proof of concept high-quality evidence is lacking, and indeed randomized controlled trials show negative outcomes in both the resectable and borderline locally advanced settings.[31,32,39,44–51]

Targeted Therapies—The Reductionist Drive

The evolution of cytotoxic therapies for the different stages of pancreatic cancer has largely been based on empirical approaches. A reductionist approach aiming to develop treatments based on targeting key pathogenic gene alterations has met with only limited success[52–66] (Table 2). The prevalence of most of the pathogenic targets range from 0.1% to 5%, and the survival advantage of these agents is a matter of only a few months. The Know Your Tumor Registry is the largest pancreatic cancer targeted therapy programme.[5] Of the 1856 patients referred, 282 (15.2%) patients had actionable mutations, but only 46 (2.5%) had matched therapy.[67] Survival since diagnosis was possible in 677 patients with a median overall survival of 1.3 years in the 488 patients with no actionable alteration, 1.5 years in 143 patients with actionable mutations who received unmatched therapy, and 2.6 years in the 46 patients who had matched therapy.[67] The targeted agent, olaparib, has now been licensed for patients with pancreatic cancer. In patients with germline mutations in the BRCA genes, olaparib can improve progression free survival from 3.8 to 7.4 months in patients that have not progressed after at least 4 months of platinum-based first-line chemotherapy.[56] The fraction of PDAC patients with druggable driver alterations will considerably increase if the novel KRAS inhibitors that target the G12D mutation present in about 40% of PDAC patients[68] are successful in clinical trials.

Mechanistic Insights into Therapy Response

PDAC Subtypes and Response to Therapy

Transcriptomic profiling of PDAC has emerged as an alternative approach to better predict prognosis and response to therapy

Table 2. FDA approved cytotoxic drugs and targeted agents for treating patients with PDAC

Reference	Cytotoxics	Stage	FDA Approval
Burris et al.[22]	Gemcitabine	Metastatic: first line	1996
Neoptolemos et al.[31,32]	5-fluoruracil/folinic acid	Adjuvant—post-surgery	NA:[α] 2004
Moore et al.[23]	Gemcitabine + *erlotinib	Metastatic: first line	2005
Conroy et al.[24]	FOLFIRINOX	Metastatic: first line	2010
Von Hoff et al.[25]	Gemcitabine + nab-paclitaxel	Metastatic: first line	2013
Wang-Gillam et al.[26]	5FU + nal-irinotecan	Metastatic: second line, post-gemcitabine	2015
Neoptolemos et al.[27]	Gemcitabine + capecitabine	Adjuvant—post-surgery	2016
Conroy et al.[28]	Modified FOLFIRINOX	Adjuvant—post-surgery	2018
Wainberg et al.[29]	5FU + nal-irinotecan + oxaliplatin	Locally advanced or metastatic: first line	2022
Reference	**Targeted Agents**	**Pathogenic Gene Target, Prevalence**	**FDA Approval**
Le et al.[51]	Pembrolizumab	**Lymphocyte programmed cell death protein 1 (PD-1) receptor, tumors with microsatellite instability (MSI-Hi) or deficient mismatch repair (dMMR), 1%–3%	2017
Marabelle et al.[52]	Pembrolizumab	¶PD-1 receptor, high tumor mutation burden (TMB ≥10 mutations/megabase), <1%	2020
Drilon et al.[53]	Larotrectinib	†Inhibitor of tropomyosin receptor kinases (TRKs), tumor NTRK1/2/3 fusions, <1%	2018
Doebele et al.[54]	Entrectinib	†TRK inhibitor, tumor NTRK1/2/3 fusions, <1%	2019
Golan et al.[55]	Olaparib	PARP inhibitor, germline BRCA1/2, maintenance, 1%–5%	2019
FDA[56]	Dostarlimab-gxly	‡PD-1 receptor, deficient mismatch repair (dMMR) tumors, 1%–2%	2021
Salama et al.[57]	Dabrafenib + trametinib	ƒBRAF/CRAF + MEK1/MEK2 inhibitors, $BRAF^{v600E}$ tumors, <1%	2022
Schram et al.[65]	Zenocutuzumab	**Neuregulin ligand (NRG1) binding to HER2: HER3 heterodimers, tumor NRG1 fusions, <1%	2022
Reference	**Emerging Targeted Agents**	**Pathogenic Gene Target, Prevalence**	
Reiss et al.[58]	Rucaparib	#PARP inhibitor. Germline or somatic BRCA1/2, or PALB2, 1%–5%	
Singhi et al.[59]	Critzotinib	ALK, 0.16% all cases, 1.6% in patients < 50-years old	
Velthaus et al.[60]	Lorlatinib	ROS1, 1%	
Strikler et al.[61]	Sotorasib	Binds to $KRAS^{G12C}$—GDP in inactive state, $KRAS^{p.G12C}$ tumors, 1%–2%	
Bekaii-Sabb et al.[64]	Adagrasib	Binds to $KRAS^{G12C}$—GDP in inactive state, $KRAS^{p.G12C}$ tumors, 1%–2%	
Heining et al.[62]	Afatinib	Pan-EGFR tyrosine kinase inhibitor in KRAS wild-type tumors with NRG1 gene fusions, <1%	

[α]NA = not applicable as 5 fluoruracil/folinic acid were generic agents not requiring FD approval, but adjuvant chemotherapy first established as proof of principle.
*Erlotinib is a small molecule tyrosine kinase inhibitor against EGFR, but the survival difference in combination with gemcitabine is a matter of only a few weeks but with considerable toxicity, so hardly used.
**This applies to agnostic solid tumors (with defined mutations but of unknown tumor type) that have progressed following prior treatment and who have no satisfactory alternative treatment options.
¶High TMB (agnostic) as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.
†Either metastatic or where surgical resection is likely to result in severe morbidity (agnostic), and who have no satisfactory alternative treatments, or whose cancer has progressed following treatment.
‡Recurrent or advanced solid tumors (agnostic), as determined by an FDA-approved dMMR test, that have progressed on or following prior treatment and who have no satisfactory alternative treatment options.
ƒUnresectable or metastatic solid tumors (agnostic), who have progressed following prior treatment and have no satisfactory alternative treatment options.
#Advanced pancreatic cancer who had received >16 weeks of platinum-based chemotherapy without evidence of resistance.

(Figure 1).[7,8,69–71] Several large-scale transcriptomic studies, performed on predominantly resected nontreated samples, have identified two broad consensus subtypes (Moffitt subtypes—reviewed elsewhere[11]) of PDAC, namely:

Classical—well-differentiated tumors that express key pancreatic-specific transcription factors (TFs) GATA6, HNF1A, and PDX1, and are associated with better outcomes; and

Basal-like—less differentiated than Classical tumors with mesenchymal characteristics, including upregulated expression of ΔNP63 and TGFβ-signaling and associated with poorest patient outcomes.

The Moffitt subtypes are prognostic for survival in patients with resected PDAC but fail to prognosticate in metastatic disease.[8,70] These subtypes may also guide therapy choice. Basal-like tumors exhibit lower response rates to chemotherapy in locally advanced or metastatic PDAC.[69,70] The COMPASS study (NCT02750657) retrospectively assessed the utility of the Classical and Basal-like subtypes for predicting survival and response to first-line mFOLFIRINOX and gemcitabine nab-paclitaxel in advanced PDAC.[71,72] Data for the intent-to-treat population showed that overall survival was almost 4 months longer for patients having a Classical RNA expression signature compared with those having a Basal-like signature. Overall survival was more than 2 months longer for those having high versus low expression of GATA6, a surrogate biomarker for the Classical subtype. Among the subset of patients given mFOLFIRINOX, patients having the Classical signature had significantly better outcomes than those having the Basal-like signature, suggesting that GATA6-low Basal-like tumors may be more resistant to mFOLFIRINOX. Based on these findings, randomized clinical trials are currently underway to evaluate expression subtyping for therapy selection in PDAC including PASS-01-Pancreatic Adenocarcinoma Signature Stratification for Treatment (NCT04469556) PANCREAS-PurIST Classification-Guided Adaptive Neoadjuvant Chemotherapy by RNA Expression Profiling of EUS Aspiration Samples (NCT04683315), and the ESPAC6 Adjuvant Trial in Patients with Resected PDAC Randomized to Allocation of Oxaliplatin- or Gemcitabine-based Chemotherapy by Standard Clinical Criteria or by a Transcriptomic Treatment Specific Stratification Signature (NCT05314998).

Despite the apparent clinical utility of the Moffitt two subtype classification scheme, recent evidence suggests that the Moffitt subtypes fail to identify overlapping but clinically distinct neoplastic subtypes in locally advanced and metastatic PDAC. Chan-Seng-Yue et al. performed de novo classification of PDAC using transcriptomic data (excluding stromal input) from 206 patients with primary resectable (stage I and II) and 111 patients with advanced (stage III and IV) PDAC and identified five neoplastic subtypes referred to as Basal-like A, Basal-like B, Hybrid (also referred to as Intermediate Co-expressors), Classical A, and Classical B.[8] This classification scheme splits the Classical and Basal-like subtypes into two subcategories and identifies tumors with Hybrid gene expression profiles that share common transcripts between the Classical and Basal-like subtypes. Based on this dataset, the Classical-like subtype was found to be more prevalent in patients with resectable PDAC whereas the Basal-like subtype was more prevalent in patients with advanced disease. Basal-like A and Basal-like B subtypes were also found to approximately distinguish metastatic disease from localized disease, with the Basal-like A subtype exhibiting greater resistance to chemotherapy.

Single-cell RNA sequencing of primary tumors and metastases has demonstrated that Classical, Hybrid, and Basal-like expression signatures segregate into distinct cell populations within the same tumor and that these subtypes represent a continuum of transcriptional states.[8,73] Williams et al. recently performed a spatially resolved single-cell assessment of Classical, Basal, and Hybrid phenotypes in resectable and metastatic patient samples using a quantitative multimarker protein panel (Classical protein biomarkers: CLDN18.2, TFF1, GATA6; Basal-like protein biomarkers: KRT17, KRT5, S100A2).[74] This analysis found that primary and metastatic samples exhibit considerable intratumoral subtype heterogeneity with very few tumors existing as purely Basal or purely Classical. While most tumors exhibited mixed Classica/Basal-like phenotypes, metastatic lesions exhibited a higher relative fraction of Basal-like to Classical cells when compared to primary tumors. Interestingly, 90% of primary tumors contained Hybrid cells with greater enrichment in metastatic biopsies. Cell–cell neighbor analysis of individual glands found that Classical, Hybrid, and Basal-like cells often co-exist in ordered chains further supporting the notion that these subtypes represent a continuum of cell states. Stratification of patient samples based on Classical, Hybrid, and Basal-like protein expression found that subtype fractional abundance was associated with survival outcomes. Patient tumors exhibiting a higher fraction of pure Classical cells were associated with better outcomes whereas patient tumors having mixed Classical and Basal cell fractions were associated with poorer outcomes.

Collectively, these findings point to increased subtype heterogeneity during disease progression with the enrichment of Basal-like and Hybrid cell populations in advanced disease. These studies also suggest that subtype plasticity may underpin disease progression and/or response to therapy with Hybrid cells acting as important transitional cell types in both resectable and metastatic PDAC. Recent ex-vivo analyses provide strong support for the role of subtype plasticity in therapy resistance.[73,75] Shalek and colleagues have demonstrated that Classical subtype Patient Derived Organoids (PDOs) treated with growth factors such as TGFβ can transition towards a Basal-like transcriptional subtype via a Hybrid or intermediate co-expressor state.[73] Importantly, TGFβ induced basal-like states were found to exhibit increased resistance to standard chemotherapies supporting earlier clinical findings. Subtype plasticity has also been observed in pancreatic cell lines treated with mFOLFIRINOX, wherein Basal-like subtypes were enriched following therapy.[75]

The reduction of PDAC tumor heterogeneity to a continuum of Classical to Basal-like transcriptional states, however, fails to recognize additional neoplastic lineages that contribute to disease progression and therapy resistance. Comparative analysis of chemo-naïve and post-treatment (chemoradiotherapy) patient tumors using single nuclei and spatial transcriptomics identified seven neoplastic lineage programmes that contribute to patient outcomes, namely Classical, Squamoid, Basaloid, Mesenchymal, Acinar-like, Neuroendocrine-like, and Neural-like progenitor.[76] This refined cellular taxonomy partitioned the Basal-like subtype into discrete Squamoid, Basaloid, and Mesenchymal lineage programmes and identified additional Acinar-like, Neuroendocrine-like, and Neural-like progenitor cell lineages. Intriguingly, cells exhibiting the Neural-like progenitor cell lineage were significantly enriched in post-treatment samples. These cells expressed several genes involved in drug efflux, negative regulation of cell death and chemoresistance (eg, ABCB1, BCL2, PDGFD, and SPP1). Moreover, neural migra-

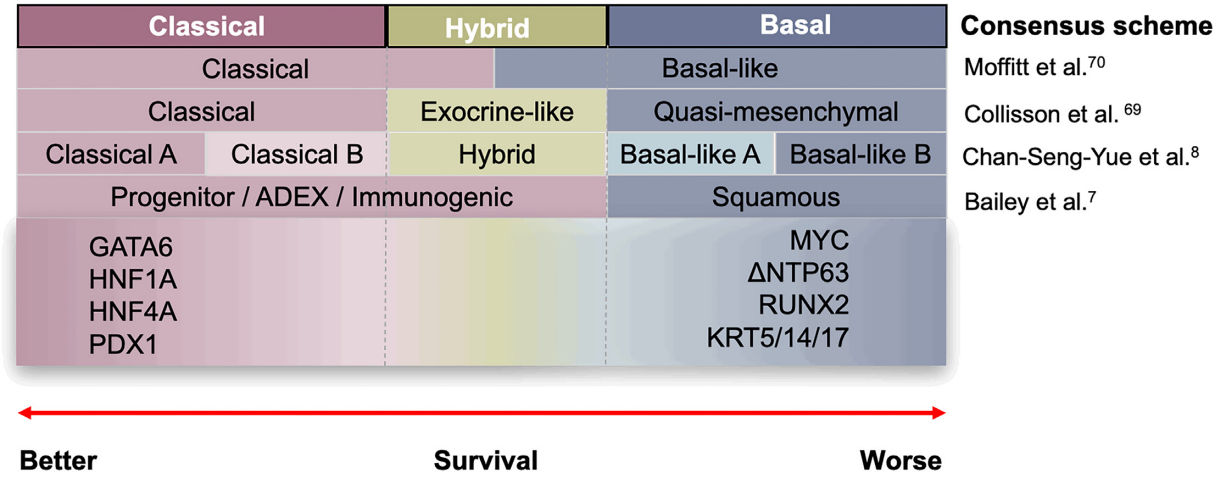

Figure 1. Transcriptomic subtype classifications and surrogate markers of PDAC. Classical tumors are associated with a better patient survival with surrogate markers: GATA6, HNF1A/4A, and PDX. Basal-like tumors have poorer outcomes with surrogate markers: MYC, ΔNTP63, RUNX2, and KRT5/14/17. Hybrid tumors show a mixed character of classical and basal with high plasticity after drug treatment.

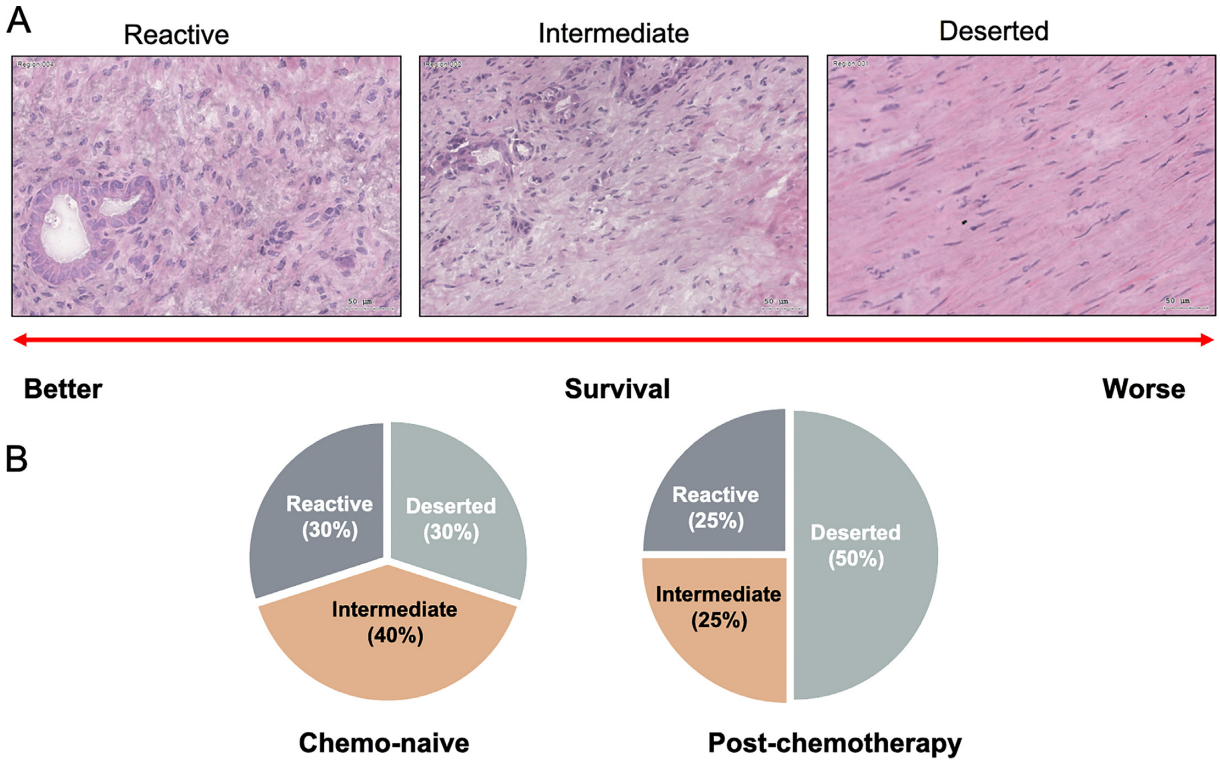

Figure 2. TME subtypes of PDAC tumors. (A) Representative images of Deserted, Intermediate, and Reactive TME subtypes. (B) TME subtype switching from a relatively balanced Deserted/Intermediate/Reactive distribution pattern to a predominant Deserted state after patients have received chemotherapy.

tion and axonal guidance genes (eg, SEMA3E, RELN and SEMA5A) were found to be expressed in Neural-like progenitors implicating these cells in tumor-neural crosstalk and possibly perinuclear invasion, which is associated with poorer patient outcomes.

Despite the stated simplicity of these findings, the genetic and nongenetic mechanisms controlling neoplastic lineage determination are complex and not well understood. Mutations in epigenetic modulators such as ARID1A and KDM6A are enriched in Basal-like tumors and are associated with metastatic progression.[7,77–79] Moreover, biallelic loss of SMAD4 with GATA6 amplification and/or CDKN2A loss with mutant KRAS allele amplifications are commonly associated with Classical and Basal-like subtypes, respectively, although not exclusively.[8] To add to this complexity, a wealth of evidence now suggests that crosstalk between neoplastic and stromal cells—cancer associated fibroblasts (CAFs) and immune cell subsets—may shape subtype identity with distinct communities of neoplastic and stromal cells (ecotypes) co-evolving within the same patient tumors.[8,74,80–82] Precisely how these ecotypes evolve during cancer progression and in response to therapy are important questions for future research.

Spatially Confined Subtumor Microenvironments (ecotypes)

Recent evidence suggests that neoplastic and stromal cells self-organize into distinct spatially confined subtumor microenvironments (sub-TMEs).[83] Based on histology, Grünwald and colleagues identified three recurrent sub-TMEs in PDAC: (i) "Deserted" containing myxoid stroma and keloid-like hyalinized collagen bundles, with thin, spindle-shaped fibroblasts; (ii) "Reactive" containing fibroblasts with enlarged nuclei and rounded morphology, inflammatory infiltrates, and few acellular components; and (iii) "Intermediate" containing intermediate levels of the Deserted and Reactive histotypes (Figure 2A). These sub-TMEs were found to co-exist within individual patient tumor samples with increased intratumoral sub-TME heterogeneity (ie, increased co-occurrence of different sub-TMEs within a single patient tumor sample) associated with advanced-stage PDAC and poor patient outcomes.

Deep molecular profiling of both Deserted and Reactive sub-TMEs revealed that while Deserted sub-TMEs were immune-poor (with sporadic T-cell/NK/B-cell signals), Reactive sub-TMEs were immune-rich comprising significant numbers of immune and stromal cell subsets including T cells, macrophages, endothelial cells, and CAFs. Higher levels of the immunosuppressive factors IDO-1 and PD-L1 and immunosuppressive cell types including FOXP3-expressing regulatory T cells (Tregs), CD11b, and CD15-expressing Myeloid-Derived Suppressor Cells (MDSCs) and CD206-expressing M2 Tumor-Associated Macrophages (TAMs) were also a feature of Reactive sub-TMEs. Moreover, Reactive sub-TMEs were enriched for diverse CAF phenotypes capable of expressing a host of proinflammatory factors including IL-1β, IL-6, TNF-a, and TGFβ. An assessment of PDAC subtype, revealed that Reactive sub-TMEs were associated with Basal-like ($KRT5^{High}/GATA6^{Low}$) phenotypes whereas Deserted sub-TMEs were associated with Classical-like ($KRT5^{Low}/GATA6^{High}$) phenotypes. Importantly, patient tumors exhibiting dominant Reactive Basal-like sub-TMEs were associated with advanced-stage PDAC and shortened disease-free survival. Encapsulating fibrosis following neoadjuvant therapy (chemotherapy and/or chemoradiotherapy) is associated with better patient outcomes in PDAC. In this study, neoadjuvant treated PDAC was associated with a higher frequency of Deserted-dominant sub-TMEs when compared to stage-matched chemo-naïve cases (Figure 2B). These sub-TMEs exhibited lower stromal immunoreactivity suggesting that chemotherapy may promote poorly vascularized, the extracellular matrix (ECM)-rich deserted sub-TMEs at the expense of aggressive Reactive sub-TME phenotypes.

Evidence derived from murine models of PDAC, demonstrate that crosstalk between neoplastic, stromal cells, and the ECM shape both neoplastic identity and localized subtumor microenvironments.[80–86] Modulation of these key cell–cell interactions or signaling pathways can modify both neoplastic state and/or localized tumor microenvironments and expose therapeutic vulnerabilities. Developing an in-depth knowledge of these tumor–stromal interactions is therefore important to unlock new and more effective therapies for PDAC (Figure 3).[11]

PDAC tumors are characterized by dense stroma, which consist mainly of host stromal cells including CAFs and an ECM.[87] The ECM is a major component of the TME that exerts mechanical and biochemical properties on tumor cells. The ECM is thought to support tumor growth by supplying nutrients, activating mechano-signaling pathways that augment oncogenic signaling pathways, and by reducing blood vessel density.[88] Fibrilla collagens such as collagen-I make up most of the ECM and can be produced by both tumor-resident CAFs and pancreatic cancer cells.[89] Recent evidence has revealed that cleavage of collagen-I by matrix-metalloproteinases can activate discoidin domain receptor 1 (DDR1)–NF-κB–p62–NRF2 signaling resulting in mitochondrial biogenesis, micropinocytosis, tumor growth, and metastasis.[86] Patient tumors enriched for intact collagen-I were associated with improved median survival compared to patient tumor enriched for cleaved collagen-I. An alternate study defined an oncogenic role for tumor-cell-produced collagen-I homodimers in PDAC.[84] Collagen-I fibers typically assemble as heterodimers (a1, a2, a1–products of the COL1A1 and COL1A2 genes); however, due to methylation of the COL1A2 gene promoter collagen-I assembles predominantly as a homotrimer (a1, a1, a1) in PDAC cells. Targeted deletion of COL1A1 in murine models of PDAC demonstrated that loss of COL1A1 significantly perturbed PDAC development and resulted in increased overall survival. Importantly, loss of COL1A1 in pancreatic cancer cells was associated with the reduced expression of the MDSCs attractant C-X-C chemokine (CXC) l5 and an increase in the expression of the T-cell attractant CXCl16. This resulted in the reduced infiltration of CD11b/GR1 MDSCs and the increased infiltration of CD4 and CD8 T cells. Treatment with an immune checkpoint inhibitor (ICI) targeting PD-1 increased overall survival suggesting that selective immunotherapies may have utility in the context of collagen-I homotrimer loss.

Expression of inflammatory cytokines such as TGFβ and TNFα have long been known to accelerate PDAC progression.[11] Recent evidence has revealed that TNFα-mediated cross-talk between macrophages and pancreatic cancer cells is important for maintaining Basal-like subtype identity.[85] Treatment of pancreatic cancer cells with TNFα was sufficient to shift Classical-like cells toward a Basal-like state. Importantly, transcription factor networks driven by master regulators BRD4 and cJUN, were found to transduce TNFα signals and to activate Basal-like gene expression programmes. Pharmacological inhibition of the BRD4/cJUN transcriptional network was sufficient to revert Basal-like cells toward a Classical state. Highlighting the remarkable interdependence of different cells within the TME, activation of BRD4/cJUN transcriptional networks by macrophage secreted TNFα induced the expression of CCL2 in neoplastic cells, a key chemokine linked to macrophage recruitment. Additional studies have shown that ablation of myeloid cells using inhibitors for colony-stimulating factor 1 receptor (CSF-1R) targeting tumor-associated macrophages or CXC chemokine receptor (CXCR) 2 targeting neutrophils in murine models can also shift Basal-like tumors toward a Classical-like state.[81,82] Importantly, these studies show that changes in neoplastic state are accompanied by increases in T-cell infiltrates and greater susceptibility to ICIs.

Halbrook et al. have demonstrated that intratumoral metabolic crosstalk between M2 macrophages and pancreatic cancer cells can promote pyrimidine-based chemotherapy resistance in PDAC.[90] M2 macrophages release a spectrum of pyrimidines including deoxycytidine (a molecular analogue of gemcitabine), which both blocks the uptake of gemcitabine by equilibrative nucleoside (ENT) and concentrative nucleoside (CNT) transporters and incorporation of gemcitabine through molecular competition at deoxycytidine kinase (DCK). Patients with a low M2 macrophage burden exhibited improved responses to gemcitabine. Moreover, targeting macrophages

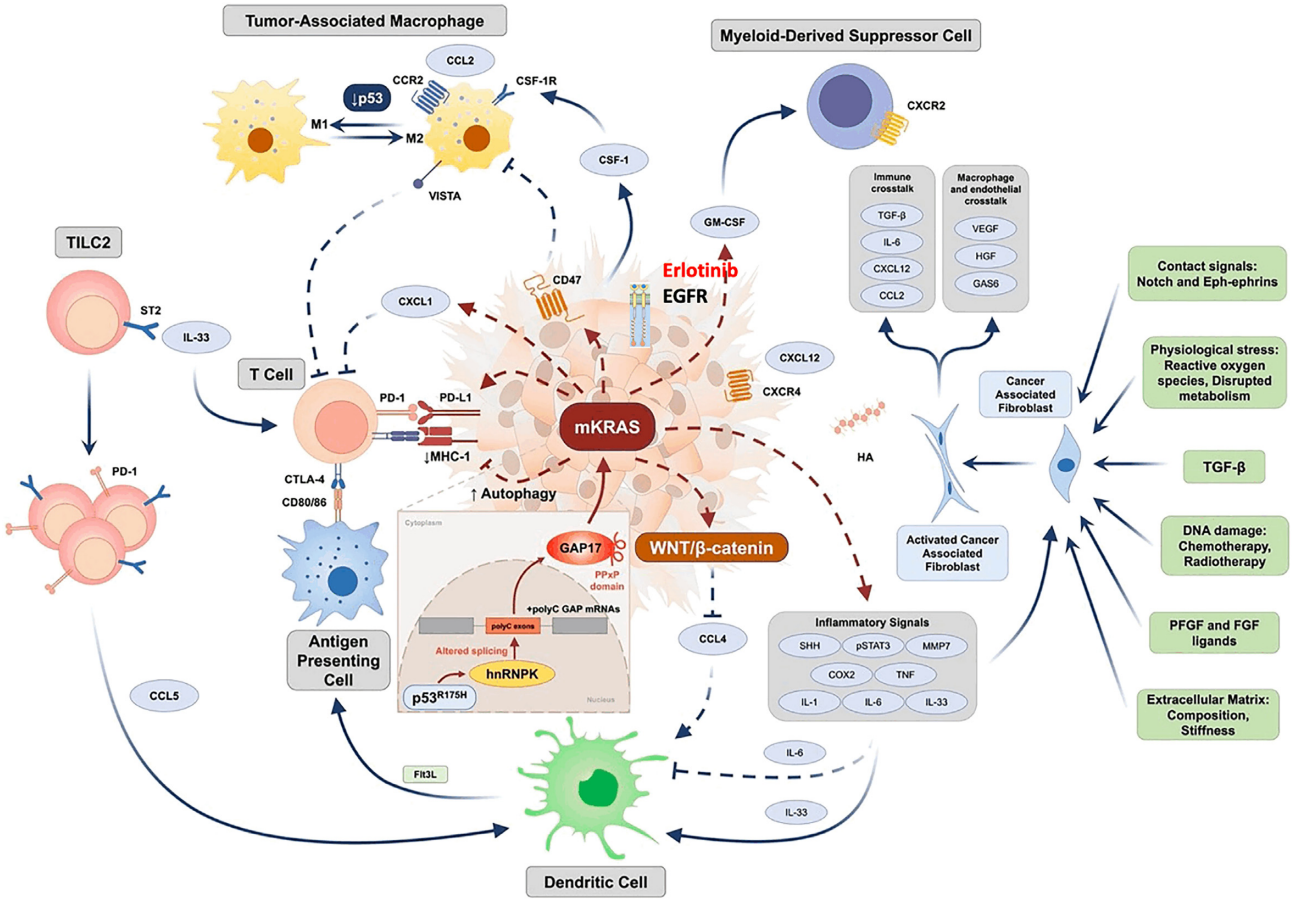

Figure 3. The PDAC TME showing tumor cell and stromal cell interactions[11]. CCL2/4/5, CC-chemokine ligand 2/4/5; CCR, CC-chemokine receptor; COX2, cyclooxygenase 2; CSF-1, colony stimulating factor 1; CSF-1R, colony stimulating factor 1 receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCL1/12, CXC-chemokine ligand 1/12; CXCR4, CXC-chemokine receptor type 4; DC, conventional type 1 dendritic cell; FGF, fibroblast growth factor; Flt3L, Fms related receptor tyrosine kinase 3 ligand; GAS6, growth arrest-specific protein 6; GM-CSF, granulocyte-macrophage colony-stimulating factor; hnRNPK, heterogeneous nuclear ribonucleoprotein K; HA, hyaluronic acid; HGF, hepatocyte growth factor; IL-1/-6/-33, interleukin-1/-6/-33; MHC-1, major histocompatibility complex 1; PDGF, platelet-derived growth factor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SHH, sonic hedgehog; ST2, suppression of tumorigenicity 2; STAT3, signal transducer and activator of transcription 3; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; and VISTA, V-domain Ig suppressor of T-cell activation.

using small molecule inhibitors (such as the CSF-1R inhibitor) increased the efficacy of gemcitabine in murine models of PDAC.

Mazzone and colleagues have demonstrated that PDAC cells express SLC4A4 a transporter that actively imports bicarbonate.[91] The accumulation of bicarbonate in PDAC cells increases intracellular pH, which in turn buffers against the accumulation of H^+ ions that are generated by glycolysis. This buffering allows glycolysis to proceed at a faster rate generating increased levels of H^+/lactate, which PDAC cells readily export into the interstitial space. Localized accumulation of lactate and increases in TME acidity establish a protumorigenic immune microenvironment. It has long been established that the glycolytic capacity is an important driver of metastatic progression and escape from immune surveillance. Increases in lactate and TME acidity are thought to modulate tumor resident immune cells in several important ways. First, CD8 cells become dysfunctional in the presence of lactate. Second, Treg cells which comprise the lactate importer SLC16A1 actively take up and metabolize lactate to increase proliferation and suppress T-cells. Thirdly, lactate induces arginase-positive macrophage phenotypes that suppress antitumor immune responses. Additionally, immune suppressive cell surface receptors such as VISTA that are expressed on immunosuppressive macrophages require low pH to engage their cognate T-cell receptor p-Selectin through a mechanism that involves protonation of receptor histidine residues. Targeted deletion of the SLC4A4 gene or pharmacological inhibition of murine pancreatic cancer cells using the small molecule DIDS decreased extracellular H^+/lactate levels and inhibited glycolysis. SLC4A4 targeting in orthotopic murine models of PDAC reinvigorated $CD8^+$ T cells and reduced immunosuppressive Treg and macrophage infiltrates resulting in reduced tumor growth and metastases. Importantly, depletion of SLC4A4 was able to overcome immunotherapy resistance to immune checkpoint inhibitors and prolong survival.

Previous efforts to broadly target the TME in PDAC have been unsuccessful. Ablation of αSMA^{pos} myofibroblastic CAFs or ablation of sonic hedgehog paracrine signaling have accelerated metastasis and reduced survival.[92] Combination therapies targeting both neoplastic and stromal cell populations may represent more effective therapeutic opportunities.

Targeting the Immune Landscape of PDAC

Immune checkpoint inhibition (ICI) has revolutionized cancer care, with up to 70 distinct USA Food and Drug Administration

label indications across more than 18 cancer types.[93] Although PDAC patients exhibiting microsatellite instability (MSI) (<1%) may benefit from ICI, single-agent, and combinations of PD-1, PD-L1, or CTLA-4 inhibitors are ineffective in patients with advanced PDAC, objective response rates being <5%.[94–96] Chemotherapy combined with ICIs, however, have been demonstrated to improve response rates in metastatic PDAC.[97,98] The randomized phase 2 PRINCE trial which tested nivolumab (anti-PD-1) in combination with gemcitabine and nab-paclitaxel (GnP) resulted in significantly higher 1-year overall survival (58%) as compared to a historical 1-year survival of 35% ($P = .006$).[98] The median progression free survival in the nivolumab plus GnP arm was 6.4 months (95% CI, 5.2–8.8), and the tumor objective response rate was 50% (95% CI, 32%–68%). Survival after nivolumab plus GnP correlated with a less repressive tumor microenvironment and higher numbers of activated, antigen-experienced T-cells at baseline. These findings point to molecularly identifiable subsets of patients that may obtain enduring clinical benefit from chemoimmunotherapy in metastatic PDAC.

Profiling of PDAC tumors indicates that as many as 20%–30% of patients exhibit moderate T-cell content, and that tumor immunogenic neo-epitopes and T-cell immunity can correlate with overall survival.[99–101] Despite the prevalence of T-cells in at least a third of patients, tumor specific T-cell responses are largely suppressed by the presence of myeloid cells in the tumor microenvironment.[102] The accumulation of tumor-modified myeloid cells derived from monocytes and neutrophils, termed MDSCs, and TAMs are associated with resistance to both chemotherapy and ICIs.[93] Preclinical efficacy studies in murine models of PDAC have demonstrated that myeloid modulators can enhance responses to both chemotherapy or immunotherapy by increasing $CD8^+$ T-cell infiltration.[81,82] The most extensively studied first-generation myeloid modulators include drugs targeting CSF-1R, C-C chemokine receptor type 2 (CCR2), and CXCR2. Clinical trials of these drugs alone or in combination with chemotherapy are ongoing in PDAC although early indications suggest that single arm Phase I and II trials in unselected populations show poor efficacy and are challenged by treatment toxicity.

Therapeutic cancer vaccines have historically been unsuccessful in PDAC[103]; however, the recent success of a vaccine targeting mutant KRAS has reignited interest in this approach.[104] KRAS mutants are found in 90% of pancreatic ductal adenocarcinomas with the G12D single acid mutation occurring in 41% of patients.[6,7,105] Mutant KRAS epitopes are presented on multiple HLA alleles and can be recognized by $CD8^+$ T-cell Receptors (TCRs) suggesting that engineered T-cell vaccines targeting mutant KRAS may elicit robust antitumor responses.[106] In the recent landmark study, a patient with progressive metastatic pancreatic cancer was treated with a single infusion of autologous T-cells that had been genetically engineered to clonally express two allogeneic HLA-C*08:02–restricted TCRs targeting mutant KRAS G12D.[104] Remarkably, this treatment induced robust and durable (>6 months) regression of visceral metastases with the engineered T-cells constituting more than 2% of all circulating peripheral-blood T-cells 6 months after cell transfer.[104] The benefit of this approach, however, is limited to the small number of patients who have the HLA-C*08:02 allele, which is present in approximately 8% of white people and approximately 11% of black people. The development of better computational tools for identifying robust candidate epitopes and improved methods of vaccine delivery may expand the utility of this approach in larger groups in patients.

Cytotoxic or metabolic stress induced by chemotherapy and/or radiation can stimulate nucleic acid sensing pathways in both tumor cells and infiltrating immune cells to trigger type-I IFN signaling (INF-α and -β).[107] Type-I IFN is required for robust immune surveillance by directly or indirectly stimulating T-cells, natural killer (NK) cells, or macrophages in the TME. Chemotherapy in combination with immune checkpoint or myeloid inhibitors has shown promise in triggering anti-tumor immune responses in PDAC, however these responses have not produced durable outcomes.[93,103,107] Tumor mutational status or changes in protein expression may underpin resistance to immunotherapy. Loss of the tumor suppressors STK11, CDKN2A, PTEN, and STING reduction in β2-microglobin are all associated with poor responses to chemotherapy and/or ICIs.[107] Homozygous deletion of chromosome 9p21.3 has been identified as a candidate biomarker of immunotherapy resistance in several cancers.[108] Tumors with deletion of 9p21.3 exhibit increased resistance to immune checkpoint inhibitors and altered immune infiltrates. The 9p21.3 locus encompasses the suppressor genes CDKN2A and CDKN2B and a cluster of type-I IFN genes. (Type-I IFN-I comprises IFNα, IFNβ, IFNδ, IFNϵ, IFNκ, IFNω, and IFNτ genes; type-II IFN comprises the IFNγ gene). Tumors with deletion of 9p21.3 exhibit either loss of CDKN2A alone or the co-deletion of the IFN gene cluster. Type-I IFN responses are critical for adaptive immune responses suggesting that co-deletion of the 9p21.3 IFN gene cluster may underpin immune checkpoint therapy resistance.

To understand the impact of 9p21.3 homozygous loss on pancreatic cancer Scott Lowe and colleagues developed MACHETE (molecular alteration of chromosomes with engineered random repeats), a CRISPR-based approach that enables the targeted deletion of large contiguous genomic regions.[108] Applying MACHETE to a syngeneic mouse model of pancreatic cancer, the Lowe group demonstrated that co-deletion of CDKN2A/CDKN2B and IFN genes combined to both activated cell proliferation and disrupt type-I IFN signaling within the TME. Disruption of type-I IFN signaling was associated with the accumulation of exhausted $CD8^+$ T-cells that express markers of terminal differentiation leading to immune evasion, metastasis, and resistance to immune checkpoint blockade.

These findings have important clinical implications for the stratification of patients receiving immunotherapy. CDKN2A copy number loss is found in around 60% of PDAC patient; however, the loss of the type-I IFN gene cluster is less well characterized. KRAS copy number gains are also associated with CDKN2A loss and basal-like transcriptional states in advanced PDAC. Many targeted trial platforms test for CDKN2A/B deletion but fail to assess loss of IFN genes within the same locus. Accordingly, future immunotherapy clinical trials in PDAC should incorporate type-I IFN status into their trial design. Preclinical development of new immunotherapies for PDAC typically utilize genetically engineered mouse models driven by oncogenic KRAS mutations and TP53 loss due to their complex TMEs.[81,82] These models, however, do not include deletions of the 9p21.3 locus. Accordingly, co-deletion of the 9p21.3 locus should be included in preclinical immunotherapy studies to develop more focused clinical trials.

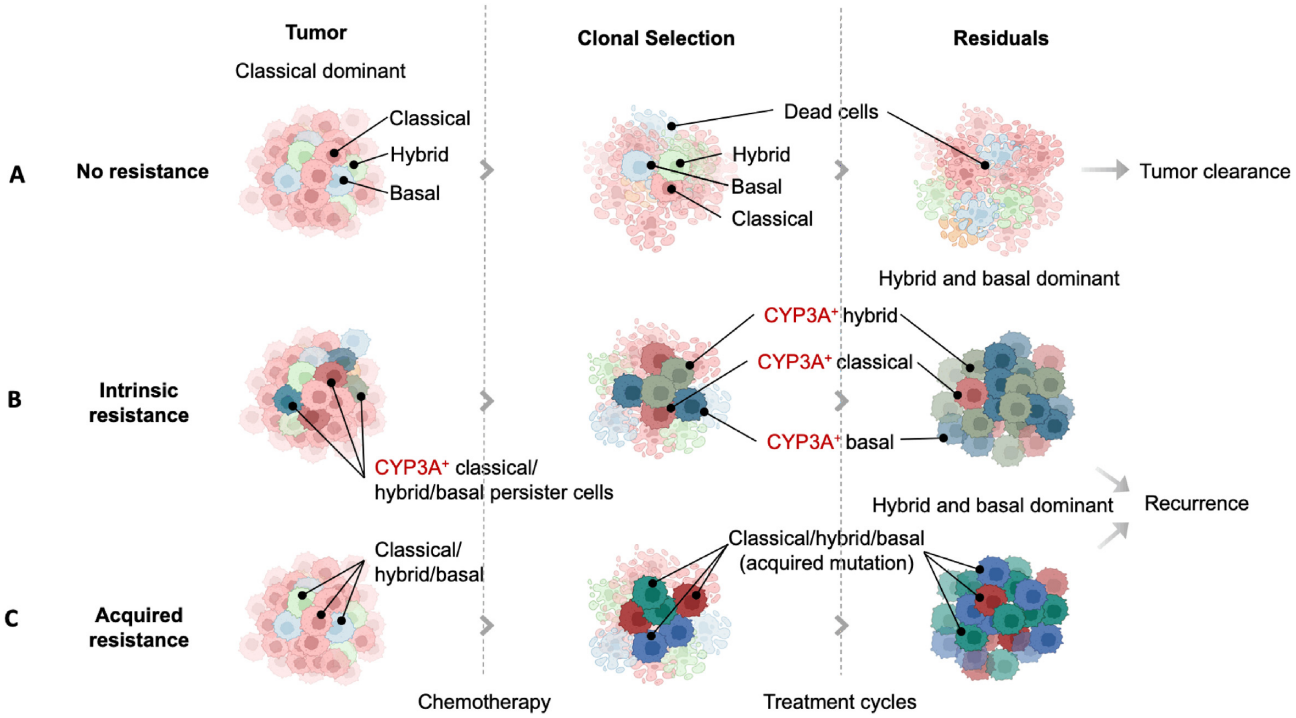

Figure 4. Drug resistance in PDAC patient after cytotoxic chemotherapy. (A) Tumor cells without drug resistance can be completely eradicated after a full (6-month) course of effective chemotherapy. (B) Tumor cell plasticity during chemotherapy results in cells with intrinsic mechanisms of cytotoxic drug resistance (such as CYP3A) to persist and become enriched as the sensitive cell types are killed. (C) Chemotherapy may drive the clonal evolution of cell types to a more Basal-like subtype with new mutations resulting in acquired resistance. Within any one tumor there will be heterogeneity with differential proportions in sensitive and resistant cell types and sub-TMEs, determining the rate of clonal evolution and ultimately survival or death.

PDAC Evolution, Drug Resistant Persisters, and Therapy

PDAC evolution is widely considered to involve the stepwise transformation of ductal epithelial from low-grade then high-grade dysplastic pancreatic intraepithelial neoplasia (PanINs) to invasive adenocarcinoma.[14] Genomic profiling of these distinct developmental stages has identified a continuum of accumulating genomic alterations. Low-grade PanINs are almost universally associated with oncogenic mutations in KRAS while increased PanIN dysplasia is associated with frequent alterations in TP53, CDKN2A, and SMAD4. Greater than 40% of intermediate and invasive adenocarcinomas are associated with complex genomic changes including increases in polyploidization and chromothripsis (a phenomenon in which a chromosome or a portion of a chromosome undergoes DNA breakages and rearrangements leading to the loss, duplication, inversion, and translocation of large segments of DNA), which contribute to significant intratumorally heterogeneity.[14]

A recent landmark study by Scott Lowe and colleagues has demonstrated that loss of TP53 (altered in 70% of PDAC tumors) enables a deterministic pattern of genome evolution in PDAC.[109] Using a mouse model of pancreatic cancer that reports sporadic loss of TP53 heterozygosity, the Lowe group traced the genomic trajectories of single cells from early dysplasia to frank PDAC. This analysis demonstrated that TP53 loss of heterozygosity initiates sequential phases of genome evolution including: (i) initial deletion of chromosomes 4 and 11 (encompassing CDKN2A and TP53 loci, respectively) as well as gain in chromosomes 5 (encompassing genes involved in PDAC proliferation or progression and TGFβ-signaling) and 6 (encompassing mutant KRAS); (ii) genome doubling; and (iii) the emergence of gains and amplifications in oncogenes, such as MYC. Importantly, evolutionary patterns observed in mice were reflected in human PDAC. Analysis of genomic data obtained from patient tumors harboring TP53-mutations demonstrated that deletion events in diploid genomes were associated with the accumulated loss of tumor suppressors on chromosomes 9p, 17p, and 18q. In contrast, diploid or polyploid genomes harboring biallelic TP53-mutations were associated with heterogeneous gains in KRAS, MYC, and GATA6—oncogenic events that drive metastasis and/or influence PDAC subtypes. Perhaps predictably, patients with polyploid genomes and biallelic TP53-mutations invariably presented with metastatic disease and had the poorest patient outcomes. Intriguingly, the observed gains and amplifications of oncogenes such as KRAS, MYC, and GATA6 in advanced PDAC mirror the emergence of Basal-like and Reactive sub-TMEs during PDAC progression strongly suggesting that these events are linked.

The ordered and deterministic evolution of PDAC toward increased genomic heterogeneity provides evidence that subclonal heterogeneity likely underpins poor responses to therapy in PDAC. In this context, the co-evolution of different subclones within the same tumor and at different stages along a deterministic path may explain why dissociated responses to therapy are common in PDAC. While these data point to a classical model of sequential genomic evolution in therapy resistance, accumulating evidence suggests that nongenetic priming or adaption contribute substantially to therapy resistance.[110] New evidence from our laboratory suggests that drug tolerant cells or "persisters" can emerge from a pre-existing subpopulation of neoplastic cells following neoadjuvant chemotherapy (Figure 4).[111] These persister-like cells adapt to chemotherapy by

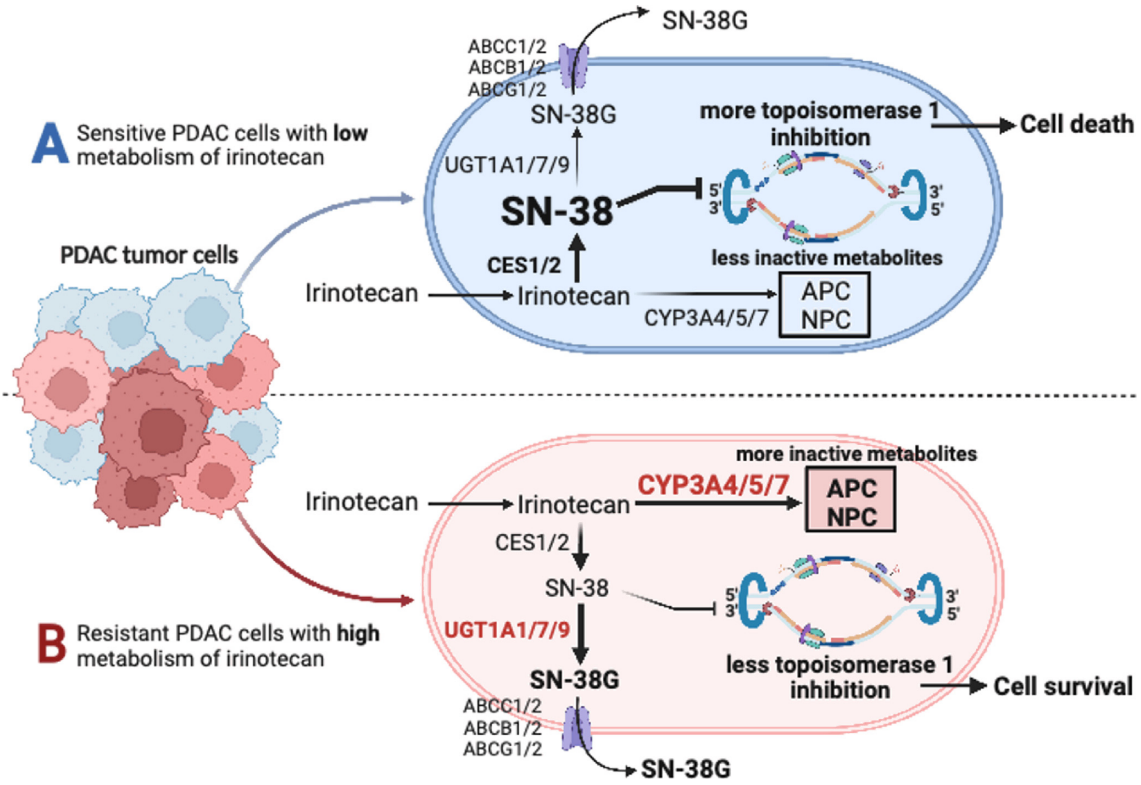

Figure 5. (A) In sensitive PDAC cells following uptake, irinotecan (a component of the FOLFIRINOX and other regimens) is converted to the active metabolite SN-38 by carboxyl esterases, which then kills the cell by inhibition of topoisomerase I. (B) In resistant PDAC cells, CYP3A isoforms 4/5/7 interfere with the metabolism of irinotecan to SN-38 by direct and indirect mechanisms resulting in cell survival. CES: carboxylesterase; CYP: cytochrome P450 isoenzymes; UGT uridine diphosphate glucuronosyltransferase isoenzymes; ABC: ATP-binding cassette transporters, the multidrug resistance-associated protein-1 is encoded by ABCC1; APC: inactive metabolite,7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyl-oxy-camptothecin; and NPC: inactive metabolite 7-ethyl-10-[4-amino-1-piperidino] carbonyl-oxy-camptothecin).

upregulating CYP3A5 and other co-expressed drug-metabolizing genes, which metabolize the prodrug irinotecan a constituent of FOLFIRINOX into nonactive forms (Figure 5). While persister cells are commonly associated with bacterial infections, there is growing evidence that analogous cell populations (alternatively referred to as "cancer stem cells" or "tumor-initiating cells") may exist in tumors. Like bacterial persister cells, cancer persister cells may enter a dormant or quiescent state, making them resistant to chemotherapy and/or chemoradiotherapy. Chemotherapy may therefore kill most cancer cells but fail to eradicate a small population of persister cells that survive and eventually repopulate the tumor. In this model, the persister cell state resembles minimal residual disease from which relapse can occur if treatment is discontinued. The identification and characterization of persister cells may therefore lead to new strategies for combating drug-resistant cancers.

Precision medicine is built on the premise that actionable genomic events can guide therapeutic decisions. The emerging picture in PDAC is that this premise is an enormous oversimplification and that complex genomic and nongenomic events underpin responses to therapy.

Evolving Therapeutic Opportunities

So, an important concept is to better understand how we can use chemotherapy and better understand the mechanisms of pancreatic cancer chemoresistance and chemosensitivity.[16] To this extent, we require a deeper understanding of the development of the tumor mass (the TME), plasticity of molecular subtypes, clonal evolution, and metastasis (Figures 1-3).[11] Different molecular subtypes are associated with differential clinical responses to chemotherapy-based regimens.[8] Stromal mediated chemoresistance to gemcitabine can be induced by TAMs by upregulation of PDAC cell cytidine deaminase, or transfer of miR-365 to PDAC cells by TAM-derived exosomes, and in mtKRAS PDAC cells with a gain-of-function mtTP53 induced chemoresistance through CAFs via NFκB/TNFα signaling leading to secretion of perlecan (Figure 3).[112–114] Chemotherapy can induce drug-tolerant persister cell phenotypes from distinct tumor cell lineages leading to tumor relapse.[11,83,111,115] The acquired drug-tolerant persister cells do not in the main involve mutations that confer therapy resistance, suggesting that alternative mechanisms, including phenotypic plasticity driven by stromal interactions, may be involved.[116] Both chemotherapy using FOLFIRINOX and chemoradiotherapy can induce a transformation to a predominant Basal-like subtype from a Classical-like subtype to associated with greater chemoresistance and reduced patient survival.[75,76,111,117] Platinum therapy after adjuvant or first-line treatment results in recurrent disease associated an increased mutational burden notably neurofibromin (NF1), AKT1, PIK3CA, and serine/threonine kinase 11 (STK11), activating MAPK/ERK and PI3K-AKT signaling, and develops from a synchronous or metachronous primaries (monophyletic and polyphyletic origins).[118] Pancreatic cancer has relatively few

neo-antigenic targets, and is embedded in an immunosuppressive cold TME, which hinders intratumoral $CD8^+$ T-cell infiltration and activation rendering single agent immunotherapies with immune checkpoint inhibitors mostly ineffective.[119] Chemoradiotherapy may also impair immunity in PDAC resulting in an increased rate of metastases whilst organs targeted with radiotherapy may also promote metastases to the radiotherapy-directed site.[120,121] Learning how to take advantage of modifying the deleterious effects of post-therapy cellular plasticity and persister cell enrichment, whilst reversing the cold immune TME is fundamental to harnessing clever clinical trials closely linked to multiple omics approaches in order to gain fundamental traction on treating pancreatic cancer. Strongly declared hypothesis driven research is required to deal with the increasing challenge of entropy of information.[122]

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Conflict of Interest Statement

C.S. declares serving on advisory boards for AstraZeneca, Bayer, Eisai, lncyte, MSD, Roche, and Servier. J.P.N. holds the position of Editorial Board Member for *Function* and is blinded from reviewing or making decisions for the manuscript. The remaining authors have no conflict of interest.

Data Availability Statement

The data underlying this article are available in the article.

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