

Molecular and Genetic Basis of Pancreatic Carcinogenesis



Which Concepts May be Clinically Relevant?

Vincent Bernard, MS^{a,b}, Jason Fleming, MD^{a,c},
Anirban Maitra, MBBS^{a,d,*}

KEYWORDS

- Carcinogenic progression • Pancreatic cancer • Genomic profiling
- Pancreatic ductal adenocarcinoma

KEY POINTS

- Carcinogenic progression of pancreatic cancer involves the stepwise accumulation of molecular aberrations from a normal cell to an invasive adenocarcinoma.
- Recent large-scale sequencing efforts have revealed novel driver genes and enriched core signaling pathways that abridge the inherent heterogeneity found in pancreatic cancer.
- By exploiting these genomic profiles, we may begin to provide new insights into patient stratification and therapeutic guidance.

Although rare (2% of cancer cases), pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in this country. In contrast with the decline of cancer-related deaths from other malignancies, the alarming increase in incidence of PDAC is projected to make it the second leading cause of cancer-related death by 2030.¹ With a 5-year survival rate of only 4%, the lethality of this disease is attributed in part to the lack of early detection and a limited armamentarium of effective therapeutic strategies.² The main oncogenic driver mutation, observed in greater than 90% of PDAC, *KRAS* (v-Kiras2 Kirsten rat sarcoma viral oncogene homolog) has also compounded its poor survival rate owing to its “undruggability,” although

^a Sheikh Ahmed Pancreatic Cancer Research Center, UT MD Anderson Cancer Center, Houston, TX 77030, USA; ^b The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA; ^c Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA; ^d Department of Pathology, The University of Texas MD Anderson Cancer Center, 6565 MD Anderson Boulevard, Room Z3.3038, Houston, TX 77030, USA

* Corresponding author. Department of Pathology, The University of Texas MD Anderson Cancer Center, 6565 MD Anderson Boulevard, Room Z3.3038, Houston, TX 77030.

E-mail address: amaitra@mdanderson.org

chemotherapeutic strategies may exist in certain targetable cases as discussed elsewhere in this paper.

Advances in next-generation sequencing technologies have allowed for a detailed insight into the genomic landscape of PDAC, to better understand how molecular alterations contribute to disease initiation and progression. In particular, dissecting the molecular events involved in the progression of PDAC from pancreatic intraepithelial neoplasia (PanINs) lesions to invasive carcinoma, are being achieved with possible implications to prognosis and targeted therapeutic approaches.

MULTISTEP PROGRESSION OF PANCREATIC DUCTAL ADENOCARCINOMA

The progression of PDAC from a normal cell to an invasive adenocarcinoma involves the accumulation of inherited and/or acquired mutations throughout a span of up to approximately 20 years (Fig. 1).³ This highlights the importance of exploiting this window of progression to develop new screening methods to provide curative surgical approaches. This progression involves the evolution of histologically recognized precursor lesions known as PanINs. As of the date of this publication, the categorization of PanIN is divided into low grade (PanIN-1A and 1B), intermediate (PanIN-2), and high grade (PanIN-3),⁴ although there is an emerging consensus in the clinical research community to move to a simplified 2-tiered classification of “low”-grade (PanIN-1 and -2) and “high”-grade PanINs (PanIN-3). This is based on observations that, although PanIN-1 and -2 lesions can be found even in the absence of cancer, PanIN-3 is almost never found without concomitant invasive neoplasia. Genetic alterations can be grouped into those that arise in precursor lesions and are usually, albeit not always, found in the concomitant PDAC, versus those that arise during subclonal evolution of the infiltrating carcinoma resulting in genetic heterogeneity.

One of the earliest genetic events involved in PDAC pathogenesis is an activating point mutation in the *KRAS* oncogene, an oncogenic driver mutation found in more

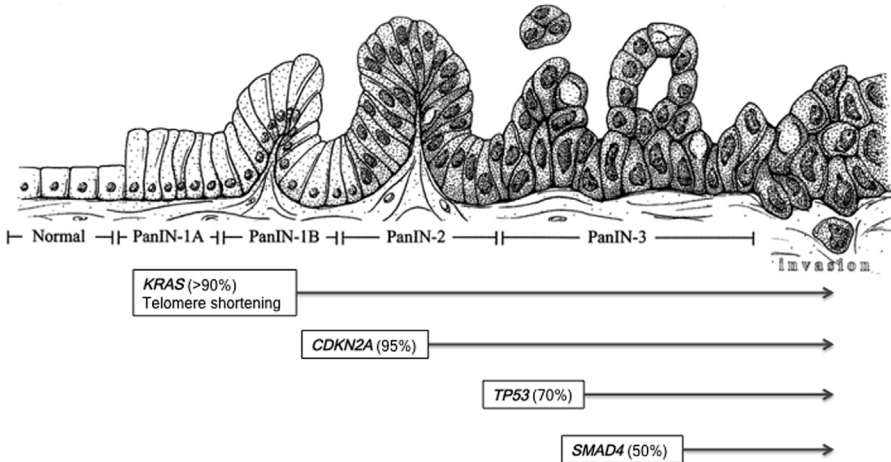


Fig. 1. A “PanINgram” illustrating our current understanding of the molecular changes in the multistep progression model of pancreas adenocarcinomas. (Adapted from Maitra A, Adsay NV, Argani P, et al. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. *Mod Pathol* 2003;16(9):909; with permission.)

than 90% of all PDACs. Subsequent genetic aberrations include inactivation of tumor suppressor genes, including *CDKN2A*, *TP53*, and *SMAD4*, which encode for p16^{INK4A}, p53, and Smad4, respectively, and contribute to the histologic evolution of these precursor lesions. Together, these 4 alterations comprise the “big 4” in PDAC, although many other recurrent somatic mutations are found in lower frequencies (5%–10%) of cases, including those that afflict particular functional pathways in the cancer cell, such as DNA damage repair or chromatin regulation.

Among the population of noninvasive precursor lesions, it is also important to recognize intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms. Although histologically distinct from the microscopic PanIN lesions, these cystic precursor lesions share similar diver mutations including point mutations in the *KRAS* oncogene⁵ and inactivating mutations in p53 and p16, with *SMAD4* loss typically not being found in IPMNs.^{6,7} Among the unique drivers found in both IPMNs and mucinous cystic neoplasms are inactivating mutations in *RNF43*, encoding for an ubiquitin ligase that has a role in WNT signaling inhibition.⁵ IPMNs also contain point mutations in the *GNAS* gene, which results in constitutively active guanine nucleotide-binding protein owing to loss of intrinsic GTPase activity preventing hydrolysis of guanosine 5'-triphosphate (GTP).⁸

KRAS

Under physiologic conditions, activation of Ras protein is induced through growth factor receptor signaling, which promotes Ras activity through transitory binding to GTP. This results in the interaction of Ras with a variety of downstream effectors that govern proliferation, cell division, survival, and gene expression such as the RAF-mitogen-activated protein kinase and phosphoinositide 3-kinase pathways.⁹ Through an intrinsic GTPase mechanism and GTPase-activating proteins, Ras can then hydrolyze GTP to GDP to inactivate itself. It is this intrinsic GTPase activity that is altered in the activating point mutation of *KRAS*, which results in an inability to hydrolyze GTP, allowing for a constitutively active protein that no longer relies on external stimuli. The most common “hotspot” mutation in the *KRAS* oncogene occurs at codon 12, followed by codon 13, and less frequently codon 61; emerging genomic data suggest that the specific codon involved might have an impact on disease prognosis, underscoring differences in Ras function.^{10,11}

TELOMERE SHORTENING

Telomeres are repetitive nucleoprotein complexes found at the end chromosomes that have a role in genomic stability by protecting against chromosomal degradation and chromosome end fusion. In PDAC, shortened telomere lengths that potentially lead to chromosomal abnormalities can be detected in early lesions such as IPMNs and are nearly universally in all PanINs.¹² Genomic instability of this kind typically leads to cell death unless cells are able to inactivate tumor suppressor mechanisms as described elsewhere in this paper.

CDKN2A

The most commonly mutated tumor suppressor in PDAC (~95%) is an inactivation of *cyclin-dependent kinase inhibitor 2A* gene (*CDKN2A*), encoding for the cell cycle checkpoint protein, p16^{INK4A}.¹³ Inactivation of *CDKN2A* in PDAC can occur via several different mechanisms, including mutation, genomic deletions, and promoter hypermethylation, resulting in epigenetic silencing.¹⁴ The encoded protein p16^{INK4A} functions

as a cyclin-dependent kinase inhibitor, specifically of CDK4 and CDK6, thereby preventing the phosphorylation of the retinoblastoma protein and blocking G1-S transition.¹⁵ Loss of this protein thus results in unregulated cell cycle transition.

TP53

Aberrations of *TP53*, which encodes for p53, are typically a later event in the multi-progression of PDAC and is mutated in up to 70% of tumors, often arising in PanIN-3 lesions.¹⁶ As the master guardian of the genome, p53 is involved in cell cycle arrest, DNA repair, blocking of angiogenesis, and induction of apoptosis in response to DNA damage or environmental stressors. Loss of this protein allows for DNA damage and external stressors to go unchecked, thereby promoting genomic instability and aberrant proliferation.

SMAD4

SMAD4 (DPC4, SMAD family member 4 gene), which encodes for the Smad4 protein, is inactivated in approximately 50% of PDACs as a late event in its progression (PanIN-3 – carcinoma).¹⁷ As a downstream effector of transforming growth factor- β , loss of SMAD4 activity leads to tumor promotion by relieving the growth inhibitory effect of transforming growth factor- β signaling.¹⁸

CLINICAL RELEVANCE OF CORE PANCREATIC DUCTAL ADENOCARCINOMA MUTATIONS

Although pancreatic cancers have been shown to harbor an average of 63 genetic mutations, the genomic landscape of PDAC is faithfully represented by these 4 genomic mutations (*KRAS*, *CDKN2A*, *TP53*, and *SMAD4*).¹⁰ The high degree of mutational concordance at these 4 loci between primary and metastatic sites of individual cancers¹⁹ suggests these are so-called founder mutations. This term describes a mutation present in all samples from a single patient, thus sharing a common ancestor, which likely originated during PanIN progression.^{3,12} Of note, PDACs continue to accumulate genetic alterations through subclonal evolution throughout their natural history, the vast majority of which are so-called passenger mutations that have little functional impact on progression, whereas a minor fraction are so-called progressor mutations, and do have a deleterious consequence on disease progression. In any case, a variable combination of the “big 4” is altered in most PDAC cases, with nearly all cancers showing at least *KRAS* mutations in conjunction with one or more of the 3 tumor suppressors. Yachida and colleagues¹⁹ describe correlations of the status of these 4 genes to disease progression, metastatic failure, and overall survival, with patients with 3 to 4 of these mutated driver genes demonstrating worse overall survival. When looking at the genes individually, there is no significant difference in outcome in patients with *KRAS* and *CDKN2A* mutations, but *TP53* and *SMAD4* mutations were evidently associated with widespread metastatic disease and worse clinical outcomes.^{20,21} In particular, *SMAD4* status in PDAC (measured using immunohistochemical expression for the Smad4 protein) is being used to provide guidance toward tailoring treatment with systemic chemotherapy, as patients with Smad4-null tumors are most likely to develop widely metastatic disease.²²

GERMLINE VARIANTS

With estimates of 10% of PDAC patients having a family history of the disease, elucidation of the PDAC genome has also contributed toward risk assessment and early detection in the context of familial pancreatic cancers.^{23,24} Among hereditary

pancreatic cancer susceptibility genes, *STK11/LKB1*, which is associated with Peutz-Jegher syndrome, is correlated with one of the highest risks of familial pancreatic cancers with approximately 132 greater relative lifetime risk.²⁵ *PRSS1* and *SPINK1* germline mutations are seen in hereditary pancreatitis families with a 50× to 80× relative lifetime risk, or 30% to 44% risk, of developing PDAC.^{26–28} *P16/CDKN2A* germline mutations, are associated with familial atypical multiple mole melanoma syndrome, which entails a 38× increased risk (17% lifetime risk) of developing PDAC.²⁹ Additional germline variants associated with increased PDAC risk are clustered into defects of DNA repair pathways. This includes members of the Fanconi anemia pathway such as *FANC-C*, *FANC-G*, and *PALB2*, whose encoded proteins interact with that of *BRCA2*, and are associated with young onset pancreatic cancer.³⁰ *BRCA1/BRCA2* mutations, which are associated with familial breast and ovarian cancers, have a 3.5× to 10× estimated relative risk.^{31–33} Lynch syndrome, caused by mutations in DNA mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2*, have an estimated 3.68% lifetime risk.³⁴ Patients with ataxia telangiectasia mutated germline mutations have also demonstrated a predisposition for PDAC.³⁵ Further elucidation of additional genes associated with familial PDAC may have implications for risk assessment and surveillance in affected family members. Detection of these germline mutations in patients is also important in the context of therapy as a way to exploit synthetic lethal interactions in the case of DNA repair pathways as described elsewhere in this paper.

CORE SIGNALING PATHWAYS IN PANCREATIC CANCER

Large-scale sequencing efforts have uncovered novel mutated genes in PDAC, as well as revealing multiple core signaling pathways that are affected throughout its carcinogenesis. In 2008, Jones and colleagues¹⁰ performed polymerase chain reaction-based exome sequencing of primary and metastatic tumors. Their data supported the role of the 4 main genetic drivers in pancreatic cancer, *KRAS*, *CDKN2A*, *TP53*, *SMAD4*, and identified genetic alterations in many other critical pathways recognized as “hallmarks of cancer,” at least some of which seem to have a prognostic influence in subsequent studies.^{11,36} The core “hallmarks of pancreatic cancer” pathways that seem to be targeted in PDAC are highlighted in **Table 1** with some salient examples of genes mutated in each pathway.

Table 1
Core hallmarks of pancreatic cancer pathways

Apoptosis	CASP10, VCP, CAD
DNA damage control	TP53, RANBP2, EP300
Regulation of G1/S phase transition	CDKN2A, FBXW7, APC2
Hedgehog signaling	GLI1, GLI3, BMPR2
Homophilic cell adhesion	CDH1, CDH2, CDH10, PCDH15
Integrin signaling	ITGA4, ITHA9, LAMA1, FN1
C-Jun N-terminal kinase signaling	MAP4K3, TNF, ATF2
KRAS signaling	KRAS, MAP2K4, RASGRP3
Regulation of invasion	ADAM11, ADAM12, DPP6, MEP1A
Small GTPase-dependent	PLXNB1, AGHGEF7, PLCB3, RP1
Transforming growth factor- β signaling	SMAD4, SMAD3, TGFBR2, BMPR2
Wnt/Notch signaling	MYC, GATA6, WNT9A, MAP2, TCF4

The altered genes in these respective pathways varied among separate patient tumors, such as the transforming growth factor- β pathway being altered by a *SMAD4* mutation in 1 patient versus a *BMPR2* mutation in another, but the pathway in itself is often altered among samples. With this new global view of the PDAC genome as a set of a specific and limited number of pathways involved, we can begin to simplify the genetic heterogeneity that is intrinsic to these tumors and develop strategies to target the physiologic effects of the mutations rather than the specific mutations themselves. By targeting key nodes involved in these pathways, such as the impaired ability to repair DNA or altered cell cycle control, we may be able to circumvent the inevitable resistance that these tumors develop after targeted gene therapies.

In 2012, Biankin and colleagues³⁷ performed next-generation sequencing of whole exomes and copy number analysis of primary resected PDAC from 142 patients, under the umbrella of the International Cancer Genome Consortium (ICGC). This study reaffirmed the core signaling pathways identified by Jones and colleagues¹⁰ and also discovered novel mutated genes in these core signaling pathways including those involved in DNA damage repair (ataxia telangiectasia mutated), which is also shown to have a role in familial PDAC. Novel gene signatures were also identified in axon guidance pathway genes (SLIT/ROBO signaling), which are known to have a role in neuronal migration and positioning during embryogenesis with potential implications in cancer cell survival, growth, invasion, and angiogenesis.³⁸ Deregulation of these axon guidance genes was shown to have a role in tumor initiation and progression in the context of PDAC. In particular, low ROBO2 or high ROBO3 expression was seen to be associated with poor patient survival. High expression of Semaphorin signaling molecules, specifically SEMA3A and PLXNA1, were also determined to be associated with poor patient survival. The ICGC team's methodology provided them the opportunity to identify potential novel drivers of pancreatic cancer, and new nodal signaling targets involving axon guidance, where therapeutics are being developed currently for neuronal regeneration after injury.³⁹ Again, we see the importance of developing therapeutics based on molecular phenotypes as further elucidation of genetic heterogeneity provides a cumbersome picture of pancreatic cancer.

In 2015, the next iteration of the ICGC PDAC dataset was published by Waddell and colleagues,⁴⁰ who performed whole genome sequencing and copy number variation analysis of 100 PDACs, and demonstrated chromosomal rearrangements that led to genetic aberrations. These structural rearrangements led to gene deletions, amplifications and fusions that are associated with driving carcinogenesis while presenting opportunities for clinical actionability and biomarkers of therapeutic response for platinum based chemotherapies. This led to the classification of PDACs into 4 subtypes based on structural rearrangement profiles (Table 2).

In addition to the classic "big 4" and alterations in genes whose products are involved in DNA maintenance, the Waddell and colleagues study highlighted the emerging importance of chromatin regulatory genes in the pathogenesis of PDAC. In particular, mutations of genes whose encoded proteins are involved in histone modification (*MLL2*, *MLL3*, *KDM6A*) and SWI/SNF genes that regulate how DNA is packaged in nucleosomes. *ARID1A* and *ARID2* emerged as a family of driver genes with unequivocal significance in PDAC. A recent whole exome study of resected PDAC patients by Sausen and colleagues³⁶ found strikingly favorable impact of harboring *MLL2* or *MLL3* mutations in the corresponding tumor, although the functional basis for this observation is still being elucidated. Inactivation of other tumor suppressors such as *ROBO1*, *ROBO2*, *SLIT2*, and *RNF43* also demonstrate the role of aberrant WNT signaling in PDAC, as well as the potential for sensitivity that these mutations may confer to WNT inhibitors.⁴¹

Table 2
Pancreatic ductal adenocarcinoma structural rearrangement profiles

Stable	Small number of structural rearrangements (<50) with defects in cell cycle characterized by aneuploidy.
Locally rearranged	Presence of intrachromosomal rearrangements classified as complex: leading to chromothripsis or breakage–fusion–bridge cycles; or focal copy number gains in genes such as KRAS, SOX9, GATA6, and potential therapeutic targets like ERBB2, MET, CDK6, PIK3CA, and PIK3R3.
Scattered	Chromosomal aberrations owing to structural rearrangements (50–200) throughout the genome.
Unstable	Widespread structural rearrangements (>200) with genomic instability pointing toward somatic or germline deleterious mutations in DNA maintenance pathways (eg, BRCA1, BRCA2, and PALB2), which suggest sensitivity to DNA damaging agents. A subset of these patients who were treated with platinum based therapy at tumor recurrence demonstrated robust or exceptional responses.

Overall, the recent series of exome studies in PDAC have suggested that a major mechanism of genomic instability and damage in pancreatic cancer involves structural variations and their potential clinical relevance. This supports the role of platinum based regimens such as FOLFIRINOX in a subgroup of patients that are both able to tolerate the regimen and contain a signature of impaired DNA maintenance pathways owing to an unstable structural rearrangement phenotype within their tumors. It may also provide a model for patient stratification for PARP-1 inhibitor therapy because current clinical trials are predominantly restricted to patient populations with BRCA1 and BRCA2 germline defects. This new model also allows for a surrogate measure of defects in DNA maintenance where there may be a larger population that may benefit from such therapies who have non-BRCA pathway gene mutations, but whose unstable tumor subtype suggests sensitivity to DNA-damaging agents.

More recently, Bailey and colleagues (Bailey P, Chang DK, Nones K, unpublished data, 2016) have used exome and RNA profiling on 450 PDAC samples in the ICGC cohort to define 4 subtypes of PDAC based on differential gene expression signatures with distinct biological underpinnings: squamous, pancreatic progenitor, aberrantly differentiated endocrine exocrine, and immunogenic (Biankin A, personal communication, 2015). Although each of these molecular subtypes is enriched in a particular histologic variant (eg, the nom de plume for squamous subtype arising from its enrichment of adenosquamous carcinomas in this subset), the intent of this expression signature is to tease out biological distinctions that might underlie PDACs that look identical at the morphologic level. Not surprisingly, as is being increasingly noted across pan-cancer profiling datasets, there exists striking molecular similarities between subtypes across cancer types than within subtypes in a single cancer. Thus, the squamous subtype of PDAC has greater similarities to the so-called basal type cancers observed in head and neck and triple negative breast cancers (characterized by an overriding p63-driven signature) than to the other 3 PDAC subtypes.

Among clinical actionability in these subtypes, MYC amplifications have been found to be associated with the adenosquamous subtype and a correlation with poor survival.¹¹ Also, appreciable differences in roles of the immune system can be identified, which may lead to exploiting immunotherapeutic strategies. In the case of the squamous subtype, a loss of cytotoxic T cells was associated with an increase in Toll-like receptors, CD4⁺ T cells, and macrophages, as well as high expression of

CTLA4 and PD1 immunosuppressive pathways. Stratification based on these subtypes may thus assist in clinical trial patient selection for therapeutics, such as PD1 and CTLA4 checkpoint inhibitors to decipher their potential role in this disease.

Many of these recent global sequencing efforts provide a biomarker-based approach to identify surrogates for prognostic and therapeutic stratification. Because most of the sequencing of data provided was performed on patients with surgically resectable primary tumors versus those undergoing recurrence or falling into the locally advanced or metastatic category, the complete picture of PDAC remains limited to a small subset of patients (~15%). Still, these efforts provide proof of concept on how measures of aberrant molecular mechanisms may inform clinical actionability using next-generation sequencing techniques.

New and promising strategies involving liquid biopsies are being developed as noninvasive methods of disease detection and monitoring.^{42,43} Specifically, through the use of circulating tumor DNA (ctDNA) that is released in the blood by primary and metastatic tumors, one can theoretically obtain a full representation of the tumor heterogeneity that is present within each patient. Sausen and colleagues³⁶ demonstrated that somatic mutation calling can be made from ctDNA in PDAC patients to determine presence of subclinical, residual, or recurrent disease after surgical resection. Detection of this ctDNA was correlated with disease progression that even predated standard computed tomography imaging by an average of 6 months, suggesting that there may be an ability to treat subclinical disease before it is overtly clinically evident based on imaging. Using ultrasensitive digital polymerase chain reaction techniques, ctDNA was detected in 43% of surgically resectable (ie, lower stage) PDAC patients at the time of diagnosis. Although this study did not examine prediagnostic samples in patients before a clinical manifestation of disease, nonetheless, it provides a potential screening approach through which high-risk patients, such as those with family history or germline mutations, can undergo noninvasive surveillance for the emergence of PDAC in time for curative surgical options.

Liquid biopsy has also shown promise in being able to genomically characterize tumors, and predict chemotherapy response and resistance using next generation techniques through both ctDNA and circulating tumor cells.^{44–46} One can thus begin to imagine how tumor evolution and the emergence of new dominant clones can be identified using these methods to guide therapeutic decisions in real time.

USING GENOMIC SEQUENCING TO GUIDE THERAPY

The genetic heterogeneity of PDAC as presented is unequivocally one of the many significant contributors to the intrinsic and acquired resistance that is characteristic of these cancers.⁴⁷ Targeting of subclonal populations will only lead to transient effects on tumor burden; thus, new strategies are required for therapeutic targeting of core pathways that are induced by founder events. By targeting convergent phenotypes that can be elucidated through genome sequencing of patient tumors, genomic information has the potential to guide individual patient therapies and outcomes.^{48,49}

In patients with familial PDAC, information of deleterious germline variants may provide some success in the cases of gene mutations in double strand break repair pathways by using therapeutics aimed at compromising additional DNA repair mechanisms such as platinum-based therapies, mitomycin C, and PARP-1 (poly [ADP-ribose] polymerase 1) inhibitors.^{50–52} By exploiting synthetic lethal approaches, which result in cancer-specific cell death through exploitation of cancer-specific molecular aberrations, one can target base excision repair through PARP-1 inhibition, leading the accumulation of chaotic DNA damage.^{53–55}

The ideal gene target in PDAC is Ras itself because it is the main oncogenic driver in more than 90% of these tumors, although efforts have so far proven ineffective.^{56,57} Synthetic lethality screens for *KRAS* have not been successful, but there have been some data suggesting possible targeting of its downstream effectors such as the MEK-ERK, MAPK, and AKT (protein kinase B) signaling pathway⁵⁸; unfortunately, recent clinical trials have shown unacceptable levels of toxicities in humans when 2 downstream Ras effectors are inhibited.⁵⁹ In the small subset of PDAC identified as harboring wild type *KRAS*, sequencing studies have found mutations in genes encoding RAS effector proteins including *PIK3CA* and *BRAF*.¹¹ In this small subset of cases, targeted therapies using BRAF and PI3 kinase inhibitors may be beneficial.

SUMMARY

Further work remains to be done to determine all key components that drive PDAC. Exploiting nodal signaling pathways versus attacking genetic heterogeneity head on may be the best strategy in overcoming the advantages pancreatic cancers have over current treatment regimens. For now, stratification of subsets of patients based on defined molecular markers into clinical trials may prove beneficial in demonstrating the effectiveness of targeted therapies in these populations.

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