Review

Advancing biomarker discovery in pancreatic ductal adenocarcinoma: Traditional approaches and emerging technologies

Saleema Mehboob Ali*

Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi Stadium Road, Karachi 74800, Pakistan

Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive solid malignancies, characterized by late-stage detection, rapid progression, and poor patient survival. Reliable biomarkers are urgently needed to improve early diagnosis, prognostic stratification, and therapeutic decision-making. Traditional approaches such as carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), and immunoassay-based methods have long supported clinical practice; however, their utility is limited by suboptimal sensitivity and specificity. Advances in molecular and analytical methodologies have broadened the biomarker landscape through next-generation sequencing, proteomics, metabolomics, and epigenetic profiling. Complementing these approaches, liquid biopsy platforms, including circulating tumor DNA, exosomes, and circulating tumor cells, as well as imaging-based radiomics and artificial intelligence-driven multi-omics integration, are enabling non-invasive, high-resolution biomarker discovery. **Objective:** This review aims to comprehensively synthesize both traditional approaches and the latest emerging technologies in PDAC biomarker discovery, emphasizing not only their underlying methodological principles but also their translational potential and the persistent challenges associated with clinical validation. **Conclusion:** By presenting a broad yet detailed methodological perspective, we trace the evolution of classical diagnostic and prognostic tools and examine how they are increasingly being integrated with innovative high-throughput and multi-omics platforms. Together, these advances underscore the pivotal role of biomarkers in enabling earlier detection, improving risk stratification, and driving the future of precision oncology.

Keywords: Pancreatic ductal adenocarcinoma, Biomarker discovery, Traditional approaches, Emerging technologies, Precision oncology

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, accounting for approximately 90% of all pancreatic cancer cases worldwide.[1-3] Despite advances in surgical and systemic therapies, the current five-year survival rate remains low at 13.3%, mainly due to late-stage diagnosis and the aggressive biological nature of the disease.[4] Nearly 80–85% of patients with PDAC are diagnosed at an advanced or metastatic stage, rendering them ineligible for curative surgical resection.[5,6]

Current clinical practice in PDAC relies primarily on serum carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA). Meta-analyses report that CA19-9 has a pooled diagnostic sensitivity and specificity of approximately 72% (95% CI: 71–73%) and 86% (95% CI: 85–86%), respectively, while CEA demonstrates notably lower sensitivity (~44%) but comparable specificity (~88%) in detecting PDAC.[7,8] These limitations are particularly pronounced in early-stage disease, reinforcing the urgent need for more accurate biomarker modalities. In cancer care, biomarkers play a pivotal role, serving diverse purposes such as early diagnosis, prognostication, selection of targeted therapies, disease monitoring, and evaluation of treatment response (Figure 1). These include a wide range of genomic and proteomic signatures, circulating tumor components, and integrative multi-omics approaches. Several reviews have provided a comprehensive synthesis

***Corresponding author:**
Saleema Mehboob Ali (saleema.mehboobali@aku.edu)

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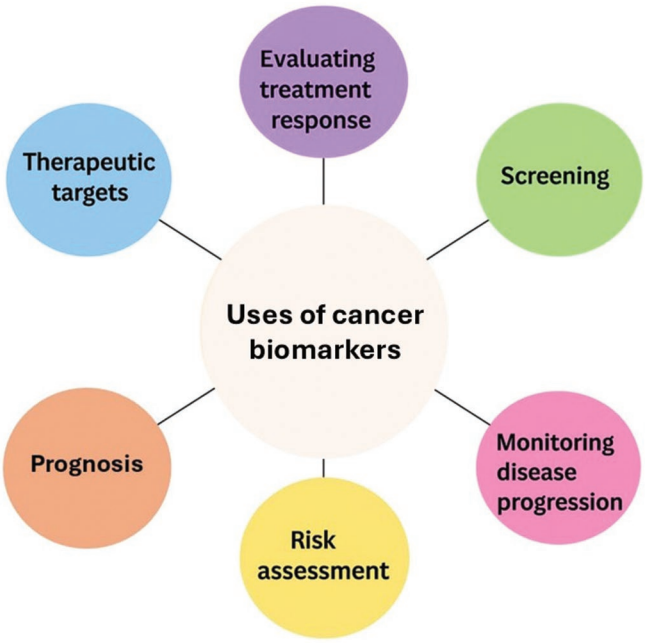

Figure 1. Clinical and translational uses of cancer biomarkers, spanning diagnosis, risk stratification, therapy selection, and disease surveillance

of candidate biomarkers.[9-14] Developing on this foundation, the present review emphasizes methodological advances that are redefining approaches to biomarker discovery and clinical implementation. An examination of established methodologies alongside emerging platforms demonstrates the capacity of technological innovation to overcome existing limitations and facilitate clinical translation.

The objective of this review is to provide a methodological synthesis of biomarker discovery in PDAC, outlining the principles, strengths, and challenges of conventional and next-generation approaches. Through this framework, we aim to contextualize their integrated potential in advancing precision oncology and improving clinical outcomes for patients with PDAC.

2. Methodology

The review was conducted through a structured literature search to identify relevant publications on biomarker discovery in PDAC. Searches were performed in PubMed, Scopus, and Web of Science databases, covering the period from January 2010 to July 2025, covering a comprehensive 15 years of scientific developments in the field. The following key terms and their combinations were used: "pancreatic ductal adenocarcinoma," "PDAC," "biomarkers," "diagnostic markers," "prognostic markers," "predictive markers," "early detection," "treatment response," "liquid biopsy," "circulating tumor DNA (ctDNA)," "circulating tumor cells (CTCs)," "exosomes," "omics," "genomic biomarkers," "epigenetic biomarkers," "DNA methylation," "microRNAs," "proteomic biomarkers," "metabolomic biomarkers," "transcriptomic biomarkers," "methodological advances," "mass spectrometry," "next-generation sequencing," "single-cell analysis," "radiomics," "imaging biomarkers," "radiogenomics," "artificial intelligence," "machine learning," "deep learning," "computational biomarkers," "precision medicine," and "biomarker discovery technologies".

Inclusion criteria were set as peer-reviewed original articles, methodological investigations, case reports, or systematic reviews that focused on biomarker discovery, validation, or translational applications in PDAC. Eligible studies employed either classical techniques, such as immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR), or emerging technologies, including next-generation sequencing, proteomics, single-cell analysis, and liquid biopsy platforms. Only articles published in English language were considered.

Exclusion criteria included conference abstracts without full-text availability, editorials, and commentaries. Research that did not directly address PDAC biomarkers or lacked methodological relevance, as well as pre-clinical studies that focused exclusively on therapeutic interventions without biomarker-related endpoints, was not considered.

3. Traditional biomarker approaches in PDAC

Among the wide spectrum of conventional biomarkers in PDAC, CA19-9 and CEA remain the most extensively investigated and clinically implemented serum biomarkers. Although the CA19-9 levels test has been approved by the U.S. Food and Drug Administration for monitoring PDAC disease progression and treatment response, its limited sensitivity and specificity significantly restrict its utility for early detection.[7,8,15] Approximately 5–10% of the population lack the Lewis antigen required for CA19-9 expression, rendering the biomarker entirely uninformative in these individuals.[16] Moreover, elevated CA19-9 levels are not specific to PDAC, owing to their occurrence in benign conditions such as cholangitis, pancreatitis, and obstructive jaundice.[17] Although CEA is valuable in gastrointestinal malignancies, its discriminatory power in PDAC is limited: meta-analyses estimate a pooled sensitivity of 44.2% (95% CI: 38.5–50.0%) and pooled specificity of 87.5% (95% CI: 82.5–91.2%), underscoring its restricted diagnostic and prognostic utility.[18] In addition, several less frequently studied biomarkers, including macrophage inhibitory cytokine-1,[19] osteopontin,[20] leucine-rich α-2-glycoprotein 1 (LRG1), and tissue inhibitor of metalloproteinases-1 (TIMP-1),[19] have also shown varying diagnostic performance in PDAC.

Evaluation of these markers has relied heavily on conventional immunoassay techniques such as ELISA and immunohistochemistry, which continue to serve as standard approaches for biomarker validation in blood and tissue

samples. While ELISA and immunohistochemistry remain accessible and cost-effective tools for biomarker evaluation, their reliance on predefined antibody-antigen interactions inherently restricts discovery to known targets.[21] Moreover, analytical and inter-laboratory variability due to antibody specificity, assay sensitivity, reagent quality, and experimental conditions continue to pose reproducibility challenges.[22,23]

The accumulated clinical and research experience with traditional biomarkers and conventional assay techniques has provided a foundational understanding of PDAC biomarker utility. Nevertheless, persistent limitations, especially in early-stage detection, patient stratification, and therapeutic personalization, underscore a critical gap in translational impact.[24] This recognition has driven a progressive shift toward molecular, multi-omics, and high-throughput platforms that offer superior resolution in characterizing the genomic, transcriptomic, proteomic, and metabolomic complexity of PDAC, thereby enhancing prospects for clinical translation.[25]

Recent multi-omics efforts illustrate this shift effectively. For instance, multimarker panels combining CA19-9, LRG1, and TIMP-1 with metabolic signatures achieved an area under the curve (AUC) of 0.924 for early-stage PDAC detection, significantly outperforming individual markers.[14] Multi-omics prognostic modeling incorporating neutrophil extracellular trap heterogeneity and TLR2 expression identified clinically relevant tumor subtypes, providing a refined survival stratification framework.[26] Furthermore, integrated multi-omics and machine-learning approaches, including data from genomics, epigenomics, transcriptomics, and metabolomics, yield molecular subtypes with robust prognostic associations, informing treatment strategies and enhancing personalized oncology.[27,28]

4. Genomic and transcriptomic approaches

The advent of high-throughput sequencing has transformed biomarker discovery in PDAC, enabling comprehensive investigation of the genomic and transcriptomic landscape. Integrated genomic studies have identified recurrent somatic alterations in driver genes (*KRAS*, *TP53*, *SMAD4*, and *CDKN2A*), underpinning PDAC pathogenesis and informing candidate predictive and prognostic biomarkers. For example, *KRAS* pathogenic mutations are observed in >90% of PDAC cases.[29,30] These pathogenic *KRAS* alterations are predominantly confined to codons 12, 13, and 61. Variants in these codons include G12D, G12V, G12R, Q61H, and G13D, collectively accounting for nearly 95% of all *KRAS*-mutated cases.[29]

Comprehensive sequencing approaches such as whole-genome sequencing, whole-exome sequencing, and targeted gene panel sequencing have further refined the biomarker landscape by capturing coding mutations, structural variants, copy-number variants, and non-coding alterations that may have clinical relevance. Large consortia-based studies have identified molecular subgroups of PDAC with distinct genomic features that carry potential diagnostic and therapeutic implications.[30-32] In addition, these technologies have also facilitated molecular profiling in underrepresented populations, in certain cases, uncovering distinct mutational patterns. For instance, while global datasets indicate that pathogenic mutations in *BRCA1* and *APC* are among the least frequently detected in PDAC, the first next-generation sequencing (NGS)-based study in Pakistani patients identified pathogenic variants in these genes, highlighting potential population-specific differences.[33]

On the transcriptomic side, bulk RNA-sequencing (RNA-seq) has been instrumental in revealing differentially expressed genes, non-coding RNA (ncRNA) signatures, and pathway dysregulation linked to tumor progression and therapy resistance. Single-cell RNA-sequencing (scRNA-seq) approaches provide high resolution of intra-tumoral heterogeneity, delineating malignant, stromal, and immune cell states and identifying rare cellular subpopulations that may serve, singly or in combination, as diagnostic biomarkers, prognostic indicators, or therapeutic targets. These single-cell studies have also clarified how treatment (*e.g.*, chemotherapy) remodels the tumor microenvironment, with implications for biomarker selection.[34,35]

Despite these advances, genomic and transcriptomic platforms face important technical and biological challenges. PDAC specimens frequently exhibit low tumor cellularity and dense stromal content, which may dilute tumor signal and reduce the sensitivity necessary for reliable genomic and transcriptomic profiling.[36] In addition, sequencing-related artifacts, batch effects, and the complexity of bioinformatic pipelines complicate the reproducibility and interpretation across cohorts.[37,38] Distinguishing actionable biomarkers from background variation in large multi-omics datasets therefore remains a major challenge for clinical implementation.[39] Genomic and transcriptomic technologies have revolutionized the discovery phase of PDAC biomarker research; however, the successful clinical integration depends on addressing issues of sample quality, assay standardization, rigorous validation, and the development of robust analytical frameworks capable of translating multi-layered molecular signals into clinically actionable biomarkers.

5. Proteomics-based approaches

Proteomics complements genomic and transcriptomic profiling by characterizing the dynamic and functional state of the proteome, including post-translational modifications

that drive cancer phenotypes. In PDAC, proteomic analyses have increasingly been used to capture disease-relevant protein signatures in tissues, cell lines, and biofluids, with direct implications for biomarker discovery and mechanistic insight.[40,41]

Mass spectrometry (MS)-based proteomics, particularly liquid chromatography-tandem mass spectrometry (LC-MS/MS) and MALDI-TOF/MALDI-MS imaging (MALDI-MSI), remains the principal technological backbone for high-throughput, sensitive protein identification and quantification in PDAC studies. These platforms support both discovery (shotgun/DIA) and verification workflows and have been applied to tumor tissue, plasma/serum, and other biofluids to delineate protein networks associated with progression, metastasis, and therapy resistance.[42-44] Galectin-3 and S100A11 were detected at approximately three-fold higher levels in PDAC tissues relative to normal pancreas through MALDI-TOF MSI, underscoring their potential as tissue biomarkers of malignancy.[45] A shotgun LC-MS/MS study with stable isotope labeling identified α-enolase, α-catenin, 14-3-3β, VDAC1, and calmodulin among 353 proteins differentially expressed in PDAC versus normal pancreas, highlighting candidates linked to tumorigenesis.[46]

Phosphoproteomics extends this capability by interrogating phosphorylation landscapes to reveal dysregulated signaling pathways and kinase activities amenable to therapeutic targeting. For instance, subtype-specific kinase activation patterns identified by tumor-intrinsic and stromal phosphoclusters (*e.g.*, PI3K/AKT, SRC/EPHA2, EGFR/MET/JAK2) highlight context-specific vulnerabilities.[47] Tyrosine phosphoproteomics in PDX models further identified EphB4 as a hyperactivated, therapeutically tractable target in over 70% of PDAC tumors.[48] In tissue analyses, dysregulated phosphorylation of proteins such as FLNA, ITGAV, and metabolic enzymes (TYMP, ACADM, ACADS, and ACAT1) provided insights into altered extracellular matrix and metabolic behaviors in PDAC.[49] Moreover, leveraging extensive phosphoproteomic datasets and INKA kinase activity scoring enabled the rational design of low-dose, multi-kinase inhibitor combinations that achieved superior efficacy in preclinical models compared to monotherapies.[47]

Targeted proteomics methods, such as multiple reaction monitoring (MRM), selected reaction monitoring, and parallel reaction monitoring, enable precise, high-throughput quantification of candidate proteins across large sample sets, bridging discovery and clinical application. For example, an MRM assay validated APOA4, APOC3, IGFBP2, and TIMP-1 in 456 clinical specimens, with a combined panel (CA19-9 + APOA4 + TIMP-1) achieving an AUC of 0.934 for distinguishing early PDAC from pancreatitis (86% sensitivity and 90% specificity).[50] Similarly, tissue and serum panels combining APOA1, APOE, APOL1, ITIH3 or APOA4, TIMP-1, and CA19-9 were robustly validated using MRM-MS, yielding high sensitivity and specificity metrics (~83–95%).[51]

Proteomic biomarker detection does not always require highly complex platforms. A study by Ali *et al.*[52] demonstrates this using immunohistochemistry to evaluate a panel of cancer stem cell (CSC) markers alongside potential therapeutic markers in PDAC tumor samples. This approach provides dual insights: it aids in understanding mechanisms underlying PDAC aggressiveness through CSC profiling, while simultaneously identifying candidate therapeutic biomarkers that could guide treatment strategies.[52] The study demonstrates that relatively accessible proteomic techniques, such as immunohistochemistry, are able to provide clinically relevant insights and connect tumor biology with potential therapeutic strategies.

Nonetheless, proteomics also faces technical and translational challenges. These include high dynamic range of proteins in plasma, pre-analytical variability, sample complexity, and the need for standardized workflows across laboratories. Addressing these issues through improved fractionation, enrichment strategies (*e.g.*, glyco/proteoform profiling), standardized quality control (QC) methods, and cross-site harmonization remains essential for reproducible biomarker discovery.

6. Metabolomics-based approaches

Metabolomic profiling, through the use of nuclear magnetic resonance (NMR) spectroscopy and MS-based approaches, has been reported to identify distinct metabolic alterations associated with PDAC in biofluids and tissues.[53,54] Proton NMR studies have demonstrated elevated plasma glucose and 3-hydroxybutyrate and reduced glutamine and histidine in patients with resectable PDAC versus healthy controls, suggesting enhanced reliance on ketogenesis and glutaminolysis.[55] Lipidomic investigations have revealed altered sphingolipid metabolism, with increased ceramide levels (notably C16:0 and C24:1) in tumor and plasma samples of patients with nodal involvement, pointing to a role in disease progression.[56] Corroborating these findings, a large-scale prospective lipidomics study reported that serum levels of various lipid species, including lysophosphatidylcholines, phosphatidylethanolamines, triacylglycerols, diacylglycerols, and fatty acids, were significantly associated with future PDAC risk, highlighting systemic metabolic reprogramming that precedes clinical diagnosis.[57]

There are several methodological challenges associated with metabolomic research. Pre-analytical variables such as fasting state, sample collection, storage, and preparation may introduce significant bias and confound inter-study

comparison. A comprehensive review emphasizes that aspects such as collection, pre-processing, transport, and freeze-thaw cycles markedly influence metabolite profiles in biofluids, underscoring the need for standardized sample handling protocols.[58] Similarly, investigations in epidemiological studies demonstrate that differences in fasting time, season, and time of day at blood draw can significantly impact the quantification of certain metabolites and should be carefully controlled or adjusted for in the study design.[59] To ensure reproducibility and robustness of data, the use of pooled QC samples is increasingly recommended in untargeted LC-MS/MS workflows. These QC samples are used to monitor technical variability and correct analytical drift, though reporting of their preparation and use often remains incomplete across studies.[60] In parallel, the adoption of standardized reference materials and SOPs as advanced by the Metabolomics Quality Assurance and Quality Control Consortium is critical to ensuring harmonization, comparability, and reproducibility across laboratories and studies.[61]

7. Liquid biopsy-based approaches

Liquid biopsy approaches, such as ctDNA, extracellular vesicles (EV)/exosomes, and CTCs, provide minimally invasive insights into PDAC biology. These modalities are increasingly being investigated for early detection, prognostication, and treatment monitoring (Figure 2). Current literature highlights substantial methodological advances across these analytes, underscoring the expanding translational relevance of multi-analyte liquid biopsy panels in PDAC research and clinical applications.[14,62]

ctDNA assays detect tumor-derived DNA fragments in plasma using highly sensitive technologies such as digital PCR (including droplet digital PCR, ddPCR) and targeted NGS panels (amplicon or hybrid-capture based) designed to call low-frequency variants.[63] In PDAC, tumor-informed and tumor-agnostic ctDNA approaches have demonstrated utility for mutation detection, minimal residual disease monitoring, and dynamic assessment of treatment response.[64] ddPCR offers improved sensitivity for hotspot mutations (*e.g.*, *KRAS*) in small-volume samples, whereas deep NGS panels enable a wider spectrum of mutation and copy-number profiling and the detection of emerging resistance alterations.[65] Cohen *et al.*[66] demonstrated that plasma-based *KRAS* mutations detected by ddPCR were present in 66% of PDAC patients, with levels correlating to tumor burden and survival outcomes. However, sensitivity is highly dependent on tumor burden and sampling site (portal vs. peripheral), with significantly reduced detection rates in localized/low-volume disease.[63,66]

Liquid biopsies represent minimally invasive approaches that allow for serial sampling and enable real-time characterization of tumor dynamics and intratumoral heterogeneity, features that are often underrepresented in single-site tissue biopsies.[62] In particular, it is useful for longitudinal monitoring of treatment response, early detection of recurrence, and interrogation of spatially heterogeneous metastatic clones. These properties make liquid biopsy attractive for PDAC, where tissue access is often limited and disease progression is rapid.[67] Despite promising applications, several technical and biological challenges constrain clinical translation. Low tumor fraction in plasma (especially in early-

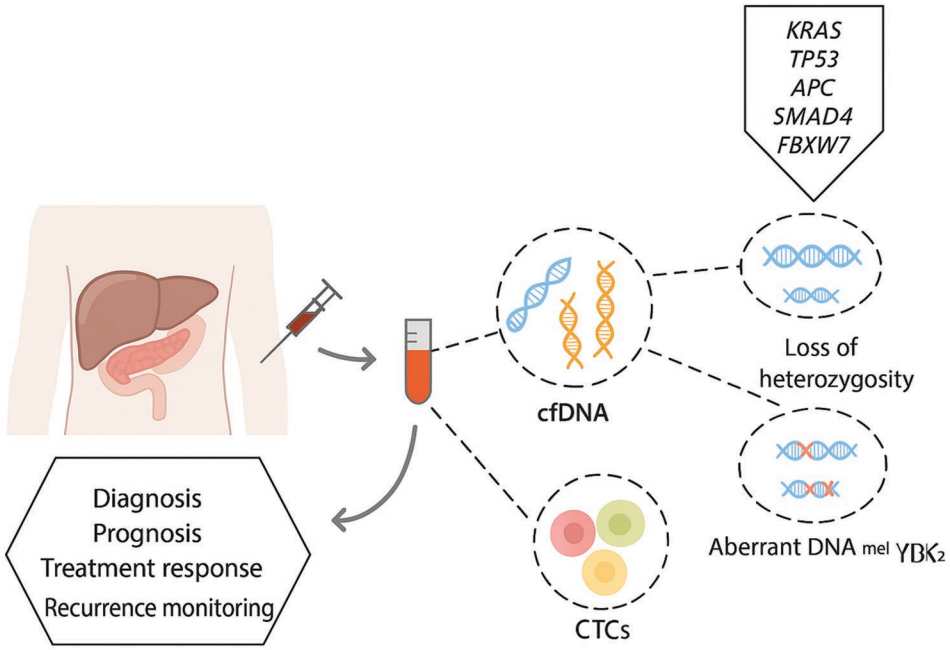

Figure 2. Liquid biopsy in pancreatic cancer. Cell-free DNA and circulating tumor cells enable the detection of epigenetic alterations, particularly aberrant DNA methylation, alongside genetic changes, supporting biomarker discovery for diagnosis, prognosis, and treatment monitoring.

stage PDAC) reduces ctDNA sensitivity; sequencing noise and bioinformatic filtering complicate detection of low-frequency variants.[68,69] EV or exosome isolation lacks standardization and can suffer from co-isolation of non-tumor vesicles; CTC assays exhibit variable recovery depending on platform and CTC phenotype.[68] Pre-analytical variables (collection tubes and processing delays), assay standardization, inter-laboratory reproducibility, and the need for large, prospective validation cohorts remain substantial barriers.[70]

Beyond technical advances, regulatory and guideline considerations are central to the clinical translation of liquid biopsy approaches in PDAC. It is important to align biomarker detection with intended clinical use such as for therapy selection, monitoring, or early detection. However, for PDAC, no liquid biopsy test is approved for routine care, and applications remain largely investigational. Implementation of multi-cancer early detection (MCED) assays in PDAC faces additional scrutiny due to their screening intent and population-level implications. In this context, real-world evidence including registry data and prospective cohort studies will be essential to establish performance across diverse populations and to inform cost-effectiveness analyses that underpin regulatory and guideline adoption.

8. Epigenetic biomarker discovery

Epigenetic modifications represent reversible, heritable changes in gene expression that occur without altering the underlying DNA sequence. Aberrations in DNA methylation, histone modifications, and ncRNA regulation are frequently observed in PDAC and have emerged as promising biomarkers for early detection, prognosis, and therapeutic response stratification.[71-73]

Aberrant DNA methylation is one of the most extensively studied epigenetic alterations in PDAC. Techniques such as bisulfite sequencing provide single-base resolution of methylated cytosines, while methylation arrays (*e.g.*, Illumina 450K and EPIC BeadChip) allow high-throughput, cost-effective profiling of CpG methylation across the genome.[74,75] In recent years, targeted methylation sequencing and enzymatic conversion protocols have enabled improved sensitivity for low-input samples, including circulating cell-free DNA (cfDNA).[76] Several methylation-based markers (*e.g.*, *ADAMTS1*, *BNC1*) have demonstrated diagnostic utility in distinguishing PDAC patients from healthy controls and individuals with benign pancreatic disease.[77]

Histone modifications, such as methylation and acetylation, alter chromatin accessibility and gene expression. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) has been the gold standard for genome-wide histone mapping, though recent low-input approaches such as Cleavage Under Targets and Release Using Nuclease and Cleavage Under Targets and Tagmentation (CUT&Tag) have improved feasibility for limited clinical samples.[78,79] In PDAC, ChIP-seq-based profiling has revealed widespread enhancer reprogramming associated with H3K27ac enrichment, which drives aberrant transcriptional networks linked to tumor progression and poor prognosis.[80] Similarly, studies have shown that loss of H3K27me3, a repressive histone mark, correlates with epithelial-to-mesenchymal transition and metastatic potential in PDAC cells.[81] Studies using CUT&Tag profiling of patient-derived organoids have identified PDAC-specific chromatin accessibility patterns that integrate with DNA methylation and transcriptomic data to define distinct regulatory subtypes.[80] Although histone-based biomarkers in liquid biopsies remain underexplored; however, integrative multi-omics studies have begun to define PDAC-specific chromatin signatures with potential clinical utility.

ncRNAs, including microRNAs (miRNAs), long ncRNAs (lncRNAs), and circular RNAs (circRNAs), regulate diverse oncogenic and tumor suppressor pathways in PDAC. Discovery platforms include qRT-PCR for targeted validation, microarrays for profiling, and RNA-seq for high-resolution transcriptomic characterization. Circulating miRNAs such as miR-21, miR-155, and miR-196a have been repeatedly implicated as diagnostic and prognostic markers.[82] LncRNAs (*e.g.*, HOTAIR, PVT1) and circRNAs (*e.g.*, circ-LDLRAD3, circRNA_100782) have also shown stability in plasma and exosomes, supporting their application as non-invasive biomarkers.[83,84] The stability of DNA methylation and ncRNA signatures in biofluids renders them promising candidates for early PDAC detection, an area of significant unmet clinical need. As reported in the literature, cfDNA methylation panels combined with protein biomarkers (*e.g.*, CA19-9) have demonstrated improved sensitivity and specificity compared to single-analyte assays.[85]

Epigenetic biomarkers offer several advantages, including tumor and tissue specificity, stability in biofluids, and complementarity with genomic and transcriptomic data. Nevertheless, unresolved technical issues limit their clinical translation. Bisulfite sequencing can cause DNA degradation, antibody specificity remains a limitation in histone assays, and ncRNA measurements are sensitive to pre-analytical variables such as sample handling. Biological confounders, including age and inflammation, can also complicate interpretation. Furthermore, while genome-wide platforms provide comprehensive coverage, their cost and data complexity may hinder clinical adoption without streamlined, validated panels.[75,76]

9. Imaging and radiomics approaches

Advancements in imaging technologies have transformed conventional radiology into a quantitative discipline capable

of generating biomarker-level information. In PDAC, imaging serves not only as a tool for diagnosis and staging but also is increasingly recognized as a source of biomarker discovery through molecular imaging and quantitative radiomic analyses. These modalities offer non-invasive, whole-tumor characterization and can complement tissue-based molecular profiling (Figure 3).

Positron emission tomography using 18F-fluorodeoxyglucose (18-FDG PET) remains the most widely used molecular imaging modality in PDAC, primarily for staging, metastatic evaluation, and prognostic stratification.[86,87] While FDG uptake reflects tumor glucose metabolism, its ability to distinguish PDAC from chronic pancreatitis is limited because of overlapping uptake patterns.[88] Hsu *et al*. reported a case of a 52-year-old patient presenting with subacute upper abdominal pain, whose computed tomography (CT) imaging demonstrated an enlarged pancreatic head with hepatic vascular encasement and PET/CT revealed increased FDG uptake, findings highly

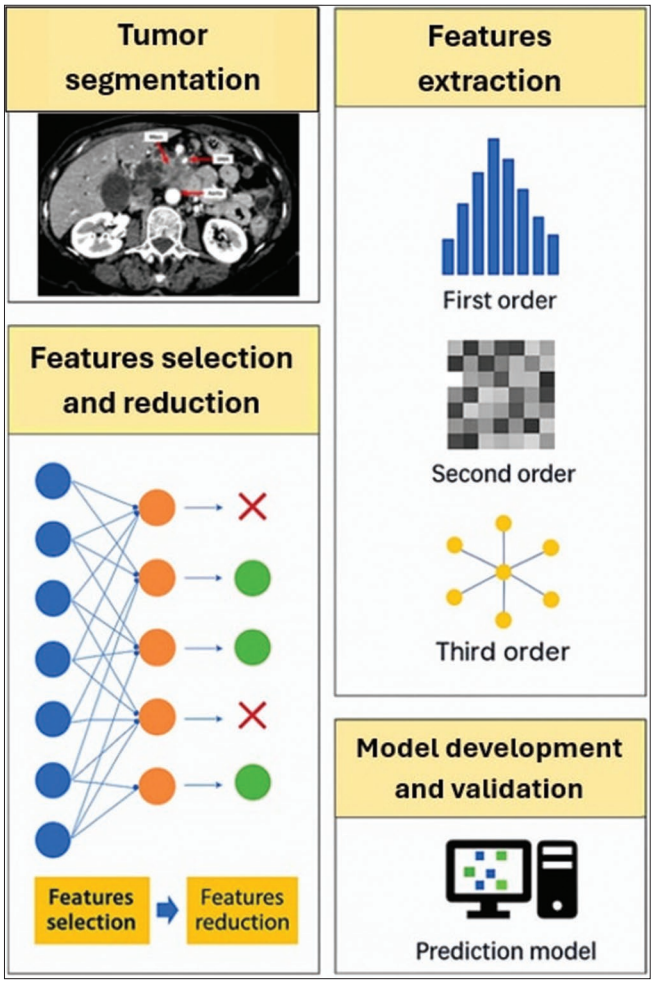

Figure 3. Workflow for radiomic biomarker discovery. The process includes tumor segmentation; extraction of first-, second-, and higher-order features; feature selection and reduction; and integration with clinical data to develop and validate predictive models.

suggestive of pancreatic cancer. However, histopathological examination following open biopsy showed inflammatory infiltration consistent with chronic sclerosing pancreatitis, and elevated serum IgG4 levels further confirmed the diagnosis.[89] In recent years, novel radiotracers directed against the tumor stroma and microenvironment have emerged as promising alternatives to conventional metabolic imaging. Among these, fibroblast activation protein inhibitor (FAPI)-based tracers (*e.g.*, 68Ga-FAPI-04) have demonstrated high tumor-to-background contrast in PDAC, reflecting the abundant cancer-associated fibroblasts in the desmoplastic stroma.[90,91] Comparative studies have reported superior lesion detection rates with FAPI PET compared to FDG PET, particularly in primary tumors and peritoneal metastases.[92]

With the integration of artificial intelligence (AI), especially deep learning, radiomics pipelines have evolved from handcrafted feature selection to fully automated frameworks capable of segmentation, feature learning, and outcome prediction. These approaches offer improved reproducibility and predictive accuracy, although they require large, annotated datasets and robust cross-validation to avoid overfitting.[93] In PDAC, CT-based radiomic signatures have been associated with histologic grade, lymph node involvement, resectability, and overall survival.[94,95] Magnetic resonance imaging (MRI)-based radiomics, although less explored due to lower availability, has shown potential in predicting response to neoadjuvant therapy and delineating tumor boundaries with higher soft-tissue contrast.[96] PET-based radiomics, particularly using FDG or FAPI tracers, can combine metabolic activity with texture-based heterogeneity to improve prognostication.[97,98]

Radiogenomics defined as the integration of imaging phenotypes with molecular data such as transcriptomics, proteomics, or mutational profiles, has emerged as a powerful strategy for multi-modal biomarker discovery. In PDAC, early studies have demonstrated correlations between specific radiomic features and molecular subtypes (*e.g.*, classical vs. basal-like), stromal content, and immune infiltration patterns.[99,100] This integration allows for non-invasive prediction of tumor biology, identification of spatially distinct molecular niches, and dynamic monitoring of therapy-induced changes. In retrospective PDAC cohorts, multi-modal modeling approaches have demonstrated enhanced predictive performance compared with single-modality strategies. Attiyeh *et al.*[101] conducted a CT-based radiogenomic analysis linking radiomic features to SMAD4 status and stromal content in PDAC, demonstrating that these imaging features were associated with survival outcomes. Iwatate *et al.*[102] demonstrated, using CT-based radiogenomic analysis of PDAC patients, that radiogenomics-predicted p53 mutations were significantly correlated with poor

prognosis (p=0.015). Another CT radiomics-based study demonstrated that incorporating MRI-derived risk scores, clinical variables, and TNM stage into a combined nomogram yielded more accurate survival predictions in PDAC compared to the conventional TNM staging system.[103] Such integrative frameworks are instrumental in PDAC, where repeated tissue acquisition is frequently limited by anatomical complexity and the aggressive nature of disease progression.

Imaging-derived biomarkers offer several unique advantages in PDAC. These include enabling whole-tumor assessment, capturing spatial heterogeneity, and allowing longitudinal monitoring without invasive procedures.[104] In addition, radiomics complements the clinical utilization of molecular biomarkers by providing functional and anatomical context, particularly for tumor-stroma interactions and microenvironmental dynamics. Importantly, imaging biomarkers can be repeatedly assessed over the disease course, enabling real-time evaluation of treatment response, recurrence, or progression.[105,106] However, several challenges limit clinical translation. First, the lack of standardization in image acquisition protocols, feature extraction algorithms, and segmentation methods leads to variability and hampers reproducibility across institutions.[107] Second, several radiomic features, particularly mathematically derived texture metrics (*e.g.*, wavelet-filtered features, gray-level run-length emphasis, cluster shade) and deep-learning "black-box" embeddings, lack clear biological interpretability, complicating downstream clinical adoption and regulatory approval.[108,109] Third, most studies to date are retrospective and single-center-based, necessitating large-scale, prospective validation in multi-institutional settings.[110] Finally, integration of imaging data into clinical workflows requires robust infrastructure, explainable AI models, and clinician training to ensure interpretability and decision support.[111]

10. AI and computational methods

Machine-learning and deep-learning approaches have accelerated biomarker discovery in PDAC across imaging, histology, and omics. CT-based radiomic features, when combined with machine learning algorithms, have demonstrated predictive value for recurrence and survival in PDAC, underscoring the potential of quantitative image-derived phenotypes as prognostic biomarkers.[112,113]

Robust multi-omics integration is central to deriving biologically relevant biomarkers. Widely used frameworks include iCluster for joint clustering across copy number and expression, Similarity Network Fusion for patient-level network integration, and MOFA/MOFA2 for factor-analysis-based latent variables that capture shared and modality-specific signals; mixOmics/DIABLO provides sparse integrative models for feature selection and supervised multi-view prediction.[114,115] These tools have been deployed broadly in cancer and are well-suited to PDAC subtyping and prognosis modeling alongside conventional molecular and histological classifications.[116]

Integration of AI with molecular data has opened new avenues in PDAC biomarker discovery. A meta-analysis of over 1,200 PDAC transcriptomes utilized non-negative matrix factorization to define six molecular subtypes (*e.g.*, tumor-specific L1–L2, stroma-specific L3–L5), each with distinct biology and survival outcomes. The investigators trained a deep-learning classifier, based on 160 subtype-specific markers, which accurately reproduced the subtype assignments in independent validation cohorts and achieved clinically relevant prognostic stratification.[117] In another study, a machine learning model using support vector machines, with leave-one-out cross-validation, identified a nine-gene secretory panel (*e.g.*, IFI27, ITGB5, CTSD, EFNA4) capable of distinguishing PDAC from healthy controls, chronic pancreatitis, and precursor lesions. This panel exhibited excellent diagnostic performance (AUC ≈ 0.95 for PDAC vs. healthy) and held prognostic utility in survival stratification.[118]

AI models also encounter extensively documented challenges that limit their translation into clinical practice. Overfitting remains a pivotal issue, especially in studies with small or single-institution datasets, where models tend to memorize patterns rather than generalize; such risks are compounded by high-dimensional radiomic data, which mandates dimensionality reduction or large training cohorts to avoid spurious associations.[119] Moreover, the lack of standardization in imaging acquisition, annotation, and processing pipelines limits reproducibility across centers. For example, disparities in scan protocols, feature extraction parameters (*e.g.*, bin-width, normalization technique, tumor subregion selection), and software implementations can substantially affect results and their comparability.[120,121] Harmonization strategies including adopting consensus feature definitions (IBSI), statistical normalization, and algorithms like ComBat to mitigate protocol- or scanner-induced batch effects are crucial but often underutilized.[122]

Finally, reproducibility remains a significant obstacle. Many radiomics workflows exhibit variability in test-retest settings, segmentation protocols, or preprocessing configurations, undermining confidence in derived biomarkers. Thorough reproducibility assessments such as repeatability testing, perturbation analysis, and transparency in parameter disclosure are essential to ensure robustness before any real-world clinical deployment.[120,123]

A summary of biomarker discovery approaches in PDAC, encompassing classical markers and innovative platforms, with emphasis on clinical utility, significance, and challenges, as shown in Table 1.

Table 1. Comparative summary of biomarker categories in PDAC, integrating methodological principles, translational relevance, and challenges

Biomarker	Examples	Methodology/ Platform	Clinical utility	Significance	Limitations/Challenges
Classical serum biomarkers	CA19-9, CEA	Immunoassays, ELISA	Diagnostic adjunct, disease monitoring, prognostic marker	First clinically used PDAC biomarkers; established benchmarks for new assays	Low sensitivity/specificity; CA19-9 not expressed in Lewis-negative individuals; elevated in benign conditions
Genetic biomarkers	*KRAS*, *TP53*, *CDKN2A*, *SMAD4*, *BRCA1*, *etc.*	PCR, NGS, targeted sequencing	Early detection (limited), prognostic stratification, therapy response prediction	Defined molecular pathogenesis of PDAC; foundation for targeted therapy research	High tumor heterogeneity; invasive sampling; limited utility in early disease
Transcriptomic biomarkers	mRNA, lncRNAs (HOTAIR, PVT1), miRNAs (miR-21, miR-34a, miR-155)	qRT-PCR, RNA-seq, microarrays	Prognostic markers, therapy response predictors	Enable functional insights into tumor biology and microenvironment	RNA instability; lack of standardized assays; small sample validation
Proteomic biomarkers	S100A, galectin-3, α-enolase, α-catenin, 14-3-3β, VDAC1, and calmodulin	Mass spectrometry, immunoblotting, and ELISA	Diagnostic and prognostic value; therapy stratification	Capture tumor heterogeneity at the protein level; closer to clinical phenotype	High cost; low reproducibility; pre-analytical variability
Metabolomic biomarkers	Altered glucose, amino acid, lipid metabolites; CA19-9+metabolite panels	NMR, LC-MS, GC-MS	Early diagnosis, metabolic reprogramming insights	Reflect systemic metabolic reprogramming in PDAC; potential for early detection	Influenced by diet, comorbidities; need large-scale validation
Liquid biopsy biomarkers	ctDNA, exosomal RNAs, CTCs	Digital PCR, NGS, microfluidics, exosome isolation	Non-invasive diagnosis, real-time disease monitoring, and treatment response	Allow longitudinal monitoring and overcome tissue biopsy limitations	Low abundance in early stages; technical variability in isolation
Epigenetic biomarkers	DNA methylation (CDKN2A, NPTX2, ADAMTS1), histone modifications, ncRNAs	Bisulfite sequencing, ChIP-seq, methylation arrays	Early detection, prognostic markers, therapy prediction	Provide stable, heritable alterations; potential for population screening	Standardization lacking; confounded by normal tissue contamination
Imaging and radiomic biomarkers	Radiomic signatures from CT, MRI, and PET	ML-based image feature extraction	Non-invasive diagnosis, prognostic modeling, treatment response prediction	Link tumor biology to imaging features; enable radiogenomic correlations	Need for multicenter standardization; limited reproducibility
AI and computational approaches	Multi-omics integration, predictive algorithms, and deep learning in imaging	AI/ML pipelines, network modeling, radiogenomics	Composite biomarker panels, patient stratification, personalized therapy	Transform large-scale heterogeneous data into clinically actionable tools	Data heterogeneity, lack of clinical interpretability, and regulatory hurdles

Abbreviations: AI: Artificial intelligence; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; ChIP-seq: Chromatin Immunoprecipitation sequencing; CT: Computed tomography; CTCs: Circulating tumor cells; ctDNA: Circulating tumor DNA; ELISA: Enzyme-linked immunosorbent assay; GC-MS: Gas chromatography-mass spectrometry; LC-MS: Liquid chromatography-mass spectrometry; lncRNA: Long non-coding RNA; ML: Machine learning; MRI: Magnetic resonance imaging; miR: MicroRNA; mRNA: Messenger RNA; ncRNAs: Non-coding RNA; NGS: Next-generation sequencing; NMR: Nuclear magnetic resonance; PET: Positron emission tomography; PCR: Polymerase chain reaction; PDAC: Pancreatic ductal adenocarcinoma; qRT-PCR: Reverse-transcription quantitative polymerase chain reaction; RNA-seq: RNA sequencing

11. Future perspectives

11.1. Integrating multi-omics, liquid biopsy, and AI for composite biomarker panels

Converging evidence suggests that combining data from genome, epigenome, transcriptome, proteome, and radiome with circulating analytes and machine-learning frameworks can yield integrated signatures with superior diagnostic and prognostic performance in PDAC.[71] cfDNA methylation and hydroxymethylation patterns, as well as fragmentomic features (*e.g.*, DELFI scores), have shown promise for detection and response monitoring, and are increasingly analyzed with AI to improve signal-to-noise at low tumor fractions.[124] Early multi-cancer platforms (*e.g.*, CancerSEEK) demonstrated that integrating cfDNA mutations with plasma proteins can detect pancreatic cancers with clinically meaningful sensitivity, providing a template for PDAC-specific panels.[125] Recent work extends this concept by applying AI to cfDNA fragmentation profiles (ARTEMIS-DELFI) to stratify outcomes and track therapeutic response in PDAC,[124] underscoring the feasibility of longitudinal, minimally invasive biomarker readouts that could be fused with tissue and imaging features in future trials.

11.2. Single-cell and spatial transcriptomics in the PDAC ecosystem

Next-generation tissue profiling may serve as a foundational framework for biomarker discovery by delineating cellular states and niches associated with progression and treatment resistance. scRNA-seq studies have delineated malignant cell continua and stromal subtypes including MHC class II-positive antigen-presenting cancer-associated fibroblasts, implicating microenvironmental programs as therapeutic

and biomarker targets.[126] Integrative single-cell plus spatial transcriptomics has revealed spatially restricted drivers, immune-stroma crosstalk, and fibroblast heterogeneity linked to prognosis and therapy exposure, enabling derivation of spatially anchored gene programs that can be projected onto bulk, liquid biopsy, or imaging readouts.[127,128] As atlases mature and become harmonized, these datasets will inform targeted panels and AI models that recognize tissue architectures and cell-state mixtures associated with risk, progression, and drug response.[129]

11.3. High-risk population and early detection

Given the low incidence of PDAC in the general population, near-term clinical impact is most likely to be realized in surveillance of high-risk individuals (HRIs). HRIs are typically defined as those carrying pathogenic germline variants, such as *BRCA2*, *CDKN2A*, *STK11*, or those with a strong family history of pancreatic cancer.[130] In this context, structured surveillance programs provide an organized framework for early detection and risk stratification. Contemporary data demonstrate that HRI surveillance is associated with earlier-stage detection and improved survival.[131] International initiatives such as PRECEDE are standardizing longitudinal imaging and biospecimen collection to catalyze biomarker discovery and validation at scale.[132] As cfDNA methylation/fragmentomics, EV-based assays, and multi-analyte blood tests advance, AI-enabled triage algorithms may integrate clinical risk factors, imaging data, and liquid-biopsy outputs. Such frameworks could support stepwise screening workflows (*e.g.*, blood-first, image-confirm) that are pragmatic, cost-effective, and suitable for prospective evaluation. Importantly, these strategies should be regarded as applicable primarily within high-risk cohorts or research settings, rather than as recommendations for general population screening, to ensure appropriate use and to avoid overextension beyond validated contexts.[124]

11.4. Population-based biomarker screening

Population-based biomarker screening for PDAC also faces a major equity gap, with most discovery and validation cohorts recruited from North America and Europe. Participation from low- and middle-income countries (LMICs) and racially or ethnically diverse groups within high-income nations remains limited. This is important because PDAC burden and outcomes vary by geography and ancestry; non-Hispanic Black populations in the U.S. have the highest incidence and mortality,[133] several indigenous communities experience worse outcomes, and PDAC burden is rising fastest in regions with a lower sociodemographic index where diagnostic capacity is constrained.[134] However, PDAC and MCED trials frequently under-enroll Black, Hispanic/Latino, Asian/Pacific Islander, American Indian/Alaska Native, and LMIC populations, and several trials omit race/ethnicity reporting altogether, limiting generalizability.[135] In U.S. PDAC trials, minorities were systematically underrepresented relative to disease burden, and fewer than 1% of patients with PDAC enroll in trials overall.[136,137]

The validation cohorts and resulting datasets for current liquid biopsy platforms reflect these imbalances. The foundational study by CancerSEEK and subsequent MCED methylation programs (CCGA, PATHFINDER) established feasibility but were dominated by White and U.S./European participants; race-stratified sensitivity in CCGA appeared broadly similar across groups but was limited by small non-White sample sizes.[138] DELFI-style fragmentomics continues to expand, but external validation in underrepresented geographies remains sparse.[124] Biological heterogeneity also intersects with equity: CA19-9, still used as a comparator endpoint, is uninterpretable in Lewis-antigen-negative individuals. However, this phenotype is observed in ~6% of White and up to ~20% of Black/African American populations, potentially biasing training labels and endpoint adjudication if cohorts are unbalanced.[7,139] A pilot study analyzing tumor samples from PDAC patients in Karachi, Pakistan, revealed the presence of pathogenic variants in the *BRCA1* and *APC* genes.[33] Interestingly, both these genes are among the least frequently reported to be altered in PDAC in global cohorts, highlighting a potentially population-specific genomic profile. These findings underscore the importance of conducting regionally representative genomic studies, as rare or under-reported alterations may have diagnostic, prognostic, or therapeutic significance that is overlooked in datasets derived primarily from North American or European populations.

Practical innovations (*e.g.*, dried-serum spot workflows) could lower barriers for LMIC screening pilots, but prospective, adequately powered, demographically diverse cohorts are essential. Priorities include (i) oversampling groups with high PDAC mortality or limited historical inclusion (Black, Hispanic/Latino, Indigenous, South Asian, sub-Saharan African, and LMIC populations); (ii) standardized capture/reporting of race/ethnicity/ancestry;[140] (iii) integration of context-specific risk factors (*e.g.*, new-onset diabetes prevalence, pancreatitis patterns, environmental exposures);[141] and (iv) head-to-head evaluation of multi-omics assays within equitable, stepwise algorithms.[142] Without these measures, population-based PDAC screening risks amplify disparities rather than reducing them. Moreover, population-specific biomarker research is essential because genetic, epigenetic, and environmental determinants of PDAC biology differ across ancestries and regions, influencing assay performance, baseline biomarker distributions, and

clinical applicability. Inclusive research ensures equitable access and advances biomarker discovery by incorporating biological diversity, resulting in more robust, generalizable, and clinically actionable screening strategies worldwide.[143]

11.5. Precision medicine in PDAC

Although currently representing a relatively small proportion of cases, actionable molecular subsets are increasingly shaping the framework for biomarker-guided therapeutic strategies. Germline *BRCA1/2* or other homologous-recombination repair alterations enable PARP-inhibitor maintenance after platinum-based chemotherapeutic drugs, as validated in the phase III POLO study.[144] Tissue-agnostic approvals, such as the use of pembrolizumab for MSI-H/dMMR tumors, extend to PDAC and underscore the importance of routine molecular testing, despite the low prevalence of these alterations.[145] Meanwhile, RAS pathway drug development is accelerating; KRAS G12C inhibitors have shown objective activity in the rare G12C-mutant PDAC subset,[146,147] and KRAS G12D inhibitors (*e.g.*, MRTX1133) have advanced from preclinical trials into early clinical evaluation.[148] Embedding these targeted options within multi-omics decision frameworks augmented by AI risk models and real-time liquid-biopsy monitoring paves the way toward adaptive, biomarker-guided treatment sequencing for individual patients.

11.6. Ethical and equity considerations in biomarker translation

As biomarker technologies advance, their ethical implications warrant careful attention. Multi-omics integration and AI-driven analytics depend on large-scale data collection, raising concerns regarding patient privacy, consent, and secure data governance.[149] Furthermore, equitable access remains a critical challenge. Most biomarker studies in PDAC are conducted in high-income settings, while underrepresented populations in LMICs face systemic barriers to inclusion in research and limited access to advanced diagnostics.[150] Without deliberate strategies, these disparities may reinforce existing global inequities in cancer outcomes. In addition, AI models trained on homogeneous datasets may perpetuate bias, leading to inaccurate predictions when applied across diverse genetic or demographic groups.[151] Ensuring fairness, transparency, and inclusivity in biomarker development will therefore be essential. Collaborative international efforts, robust ethical frameworks, and policies that prioritize diversity in research participation are critical to realizing the full promise of precision medicine in PDAC.

11.7. Emerging biomarker modalities in PDAC

In addition to the methodological domains discussed, several emerging biomarker strategies for PDAC warrant acknowledgment, though they remain underexplored and require further validation. Urine-based proteomic and peptidomic panels have shown preliminary promise as non-invasive detection tools, offering potential advantages in accessibility and patient compliance.[152,153] Glycomic and glycoproteomic approaches, particularly analyses of CA19-9 glycoforms and aberrant glycosylation patterns, are being investigated to improve specificity over conventional assays.[154] The gut and oral microbiome have also been implicated in PDAC risk and progression, with compositional signatures proposed as adjunctive biomarkers.[155,156] Moreover, novel computational approaches such as endoscopic ultrasound-radiomics and AI-driven digital pathology analysis are emerging as complementary tools to enhance diagnostic precision.[157] While these directions are currently less mature compared to established biomarker modalities, they represent important avenues for future research and hold potential for integration into multi-modal early detection strategies.

12. Conclusion

Advances in genomics, transcriptomics, proteomics, metabolomics, epigenetics, imaging, and computational sciences are transforming biomarker research in PDACs. While individual modalities have revealed important insights, their integration through AI and multi-omics pipelines holds the greatest promise for generating clinically actionable signatures. Importantly, liquid biopsy approaches and next-generation tissue profiling are paving the way for non-invasive diagnostics and precision-guided therapies. Nonetheless, significant challenges remain, including issues of reproducibility, data standardization, and the limited representation of diverse populations in biomarker studies. Addressing these gaps will require coordinated, interdisciplinary efforts that bring together clinicians, biologists, computational scientists, and public health experts. Such collaboration is essential to accelerate translation from discovery to practice and to ultimately improve early detection, risk stratification, and survival outcomes in PDAC.

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