PORTFOLIO SAMPLE

How This Document Came to Be

About this sample. A principal investigator (PI) affiliated with a university medical school, facing a deadline-bound request to create an academic review, came to us for a detailed pre-manuscript survey of the literature and current opinion pertinent to their topic: pancreatic ductal adenocarcinoma (PDAC).

We began by discussing the team's goals and assessing the available tools and public resources, including our own. We also considered systems developed by other teams. When another tool is better suited to a particular task, we recommend it.

In this case, our research-writing and data-mining system was a good fit. Working with the client, we turned the initial idea into a focused research brief and developed the prompt reproduced below. The system generated a "writing kit", which our specialists then checked and revised in consultation with the client. We verified the bibliographic references and performed an internal review tracing the quantitative claims to their sources.

The brief we developed with the client

> "Create a comprehensive and deep academic review on the following topic: 'Immune Mechanisms and Therapy in Pancreatic Cancer: Murine Models and Human Trials'."

A note on deliverables. This PDF document itself is a small part of our full research output. The client deliverables include PRISMA-compliant literature search strategies, PubMed retrievals, writing blocks that the client may use for reconstruction and revision while catering to different reporting formats, a reference database compatible with major bibliographic software, Python code for data extraction and analysis, and more.

Disclaimer. Our team has verified the bibliographic references and checked the quantitative claims against their cited sources. Nothing in this document constitutes medical advice.

Immune Mechanisms and Therapy in Pancreatic Cancer: Murine Models and Human Trials

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) stands out as one of the most lethal malignancies, with a five-year survival rate lingering around 10% despite current standard therapies (Guo et al., 2023; Ju et al., 2024). This dismal outcome is largely attributable to the uniquely hostile tumor microenvironment (TME) of PDAC, which effectively blunts antitumor immune responses. PDAC tumors are characterized by a dense desmoplastic stroma and myriad immunosuppressive components that together create an “immune-privileged” niche around the cancer (Guo et al., 2023). Indeed, while immune checkpoint inhibitors have revolutionized the treatment of other solid tumors, they have demonstrated virtually no clinical benefit in PDAC to date, underscoring the profound immune evasion at play in this disease (Byrne et al., 2021). Only in exceptionally rare cases—notably the ~1% of PDAC patients whose tumors are mismatch-repair deficient—have durable responses to PD-1 blockade been observed, highlighting how unusual it is for PDAC to be susceptible to immunotherapy in its natural state (Le et al., 2017; Byrne et al., 2021).

In the sections that follow, we explore in detail the **immune-evasion mechanisms** that underlie PDAC’s resistance to immunotherapy and how these mechanisms have been elucidated using state-of-the-art murine models. We then examine the landscape of **immune-based therapeutic strategies**—from checkpoint inhibitors to vaccines and adoptive cell therapies—that have been tested in preclinical PDAC models and advanced into human clinical trials. Emerging directions in this field focus on subtype-resolved targeting of cancer-associated fibroblast (CAF) and myeloid states, metabolic checkpoint blockade targeting CD39/CD73, IDO, and hypoxia pathways, and multi-modal priming strategies. By highlighting both the advances achieved and the setbacks encountered, we aim to clarify how insights from mouse models are guiding new treatment approaches in patients, and what challenges remain in making immunotherapy a viable cornerstone of PDAC management.

2. Immunosuppressive Tumor Microenvironment in PDAC: Mechanisms of Immune Evasion

Central to these evasion mechanisms is the complex, immunosuppressive TME that enables the tumor to escape immune surveillance even in the presence of an intact immune system. Fibroblast-rich stromal tissue envelops PDAC lesions and constitutes a large fraction of the tumor mass; this barrier enforces an "immune-excluded" phenotype that thwarts effective T-cell infiltration (Guo et al., 2023; Saka et al., 2020). Cancer-associated fibroblasts (CAFs) are the dominant stromal component and play a central role in this evasion. Recent research has identified distinct CAF subpopulations—myofibroblastic, inflammatory, and antigen-presenting—that enforce immune exclusion through unique mechanical and biochemical programs (Öhlund et al., 2017; Elyada et al., 2019) (Figure 1).

Myofibroblastic CAFs (myCAFs), marked by high \alpha-smooth muscle actin (\alphaSMA) expression, concentrate immediately adjacent to neoplastic cells and deposit a dense extracellular matrix that increases tissue stiffness, creating a physical barrier to lymphocyte infiltration (Öhlund et al., 2017). Beyond mechanical exclusion, myCAFs secrete the chemokine CXCL12, which binds to CXCR4 on T cells and effectively corrals these lymphocytes in the stromal compartment away from tumor cell islets (Feig et al., 2013). As discussed in Section 3.1, this immune-excluded phenotype is faithfully recapitulated in genetically engineered mouse models like the KPC strain.

By contrast, **inflammatory CAFs (iCAFs)** reside more distal to the cancer nests and adopt a secretory phenotype driven by tumor-derived inflammatory signals (Öhlund et al., 2017). iCAFs secrete high levels of interleukin-6 (IL-6), IL-11, and leukemia inhibitory factor (LIF). IL-6 from these fibroblasts activates STAT3 signaling in immune cells and is strongly linked to the recruitment of **suppressive myeloid cells**, such as Gr1^+ myeloid-derived suppressor cells (MDSCs) and M2-polarized tumor-associated macrophages (TAMs) (Öhlund et al., 2017). These TAMs and MDSCs form a synergistic network that paralyzes anti-tumor T-cell responses by releasing mediators like IL-10, transforming growth factor-\beta (TGF-\beta), arginase-1, and prostaglandin E2 (Yang et al., 2021; Siret et al., 2020). These factors directly suppress the proliferation and cytotoxicity of CD8^+ T cells and natural killer (NK) cells while fostering the development of FoxP3^+ **regulatory T cells (Tregs)** (Yang et al., 2021). Adding further complexity, **antigen-presenting CAFs (apCAFs)** express MHC class II and CD74 but lack co-stimulatory molecules like CD80 or CD86, potentially acting as a stromal “dead end” that renders CD4^+ T cells anergic or tolerogenic (Elyada et al., 2019).

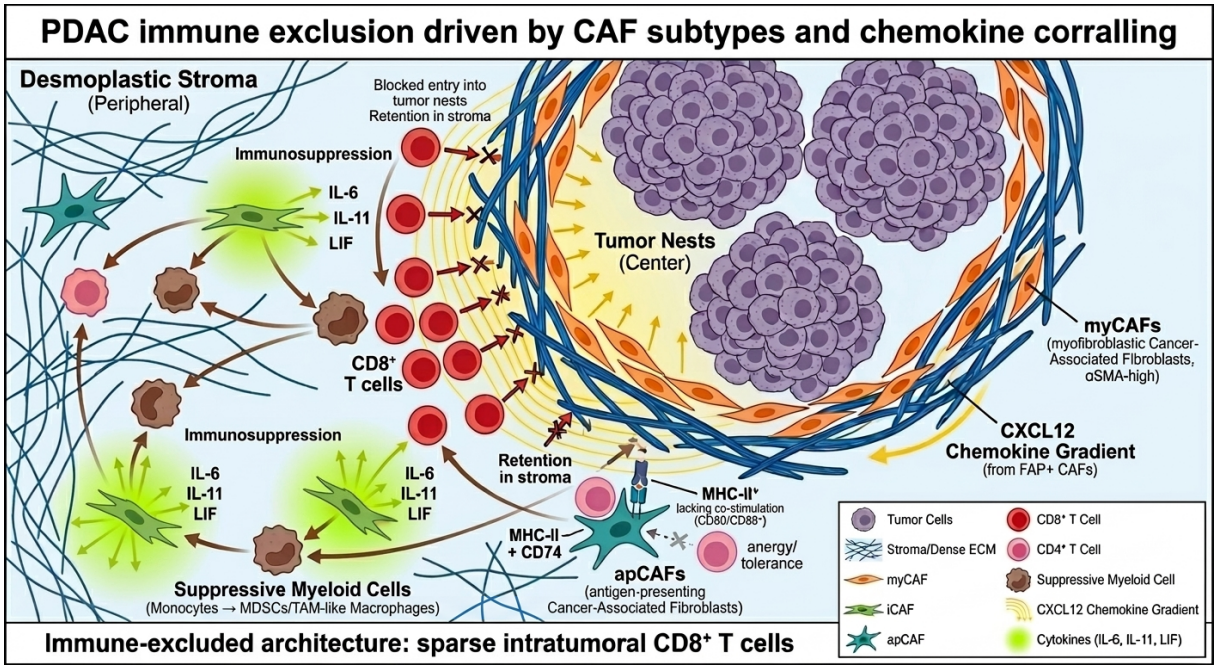

Figure 1. PDAC immune exclusion driven by CAF subtypes and chemokine corralling. *Schematic of the spatial organization that excludes cytotoxic T cells from PDAC tumor nests. Tumor-cell nests (center) are encased by a dense desmoplastic stroma. Juxtatumoral myofibroblastic CAFs (myCAFs; αSMA-high) deposit a stiff extracellular matrix that forms a physical barrier to lymphocyte infiltration (Öhlund et al., 2017). FAP-expressing CAFs (which overlap with myCAFs) secrete CXCL12, establishing a chemokine gradient that engages CXCR4 on CD8+ T cells and corrals them within the stroma, away from tumor islets (Feig et al., 2013); the result is sparse intratumoral CD8+ T cells. More distal inflammatory CAFs (iCAFs) release IL-6, IL-11, and LIF (Öhlund et al., 2017); IL-6–STAT3 signaling is associated with recruitment and M2/suppressive polarization of myeloid cells (Gr1+ MDSCs and TAMs), which dampen CD8+ and NK-cell function. Antigen-presenting CAFs (apCAFs) express MHC class II and CD74 but lack CD80/CD86, so antigen presentation to CD4+ T cells is predicted to drive anergy/tolerance (Elyada et al., 2019). Net architecture: an immune-excluded tumor with effector T cells retained in the stroma.*

Beyond these stromal barriers, FoxP3^+ Tregs represent another prominent immunosuppressive component, present even at early PanIN lesions and increasing as tumors progress (Mota Reyes et al., 2022). In both human PDAC samples and compatible mouse models, FoxP3^+ Tregs accumulate to high levels within tumors, often outnumbering conventional CD4^+ T cells and aligning with a paucity of intratumoral CD8^+ cytotoxic T lymphocytes (Zhang et al., 2020). This inverse relationship suggests that Treg-rich PDAC tumors tend to be “T cell–excluded,” and indeed

a high ratio of FoxP3^+ Tregs to granzyme B^+ effector T cells in resected PDAC is associated with significantly shorter patient survival (Hwang et al., 2016). Mechanistically, these Tregs suppress antitumor immunity by releasing immunomodulatory cytokines—expanding upon the IL-10 and TGF-\beta signaling initiated by myeloid cells—and by direct cell-contact inhibition of dendritic and T cells via surface molecules like CTLA-4, which sequesters costimulatory ligands (Saleh & Elkord, 2020). However, an unanswered question concerns which specific PDAC Treg subsets—such as IL-33–responsive versus “conventional” FoxP3^+ cells—are causally responsible for CD8^+ exclusion as opposed to functional exhaustion (Pu et al., 2025).

Concomitant with Treg-mediated immunosuppression, PDAC tumors often exploit T-cell checkpoint pathways to induce exhaustion of any infiltrating cytotoxic T cells. In particular, PD-L1 is upregulated on pancreatic cancer cells and within the tumor stroma (notably on TAMs), providing ligands that engage PD-1 receptors on T cells and blunt their activity (Tsukamoto et al., 2019). Many PDAC patient tumors with high PD-L1 expression show especially poor clinical outcomes, reflecting the profound immune escape conferred by this pathway (Tsukamoto et al., 2019). At the cellular level, chronically stimulated PD-1^+ CD8^+ T cells in PDAC display hallmarks of exhaustion—diminished proliferation and effector function—due to PD-1/PD-L1 signaling that interferes with T-cell receptor and co-stimulatory pathways (Saka et al., 2020). Spatially resolved analyses of human PDAC have reinforced this paradigm: the proximity of PD-1–expressing CD8^+ T cells to PD-L1^+ macrophages within tumors strongly correlates with attenuated T-cell activity and worse patient survival (Yang et al., 2023). This spatial correlation highlights a major controversy in the field: whether PD-1 blockade fails primarily because CD8^+ T cells are physically scarce and spatially segregated from tumor cells by CAF-generated barriers, or because they are irreversibly exhausted by these PD-L1^+ macrophage niches and parallel checkpoints like TIGIT/CD155 (Freed-Pastor et al., 2021).

The combined presence of FoxP3^+ Tregs and PD-1/PD-L1 checkpoint engagement creates a multilayered suppression of cytotoxic T cells. Tregs and PD-L1^+ myeloid cells often co-localize in PDAC, forming an immunosuppressive niche that continuously inactivates incoming effector lymphocytes (Pu et al., 2025). As a result, even tumors with antigenic potential remain “cold” or non-immunogenic because tumor-specific T cells are either kept physically at bay or rendered anergic/exhausted upon entry (Ho et al., 2020). Notably, recent evidence from murine PDAC models indicates that this suppression is highly robust and compensatory. Depleting Tregs in an autochthonous KPC model increased intratumoral T-cell infiltration but, rather than controlling disease, reprogrammed cancer-associated fibroblasts (via loss of Treg-derived TGF-\beta and tumor-restraining myofibroblastic CAFs) and expanded immunosuppressive myeloid cells, thereby accelerating—not slowing—pancreatic carcinogenesis (Zhang et al., 2020). This raises the question of how timing and disease stage dictate the paradoxical acceleration seen after Treg depletion in advanced disease. Such findings underscore a redundant network of immune evasion: when one suppressive mechanism is removed, PDAC can activate others, such as the CD155/TIGIT pathway, to maintain immune tolerance (Freed-Pastor et al., 2021) (Figure 2).

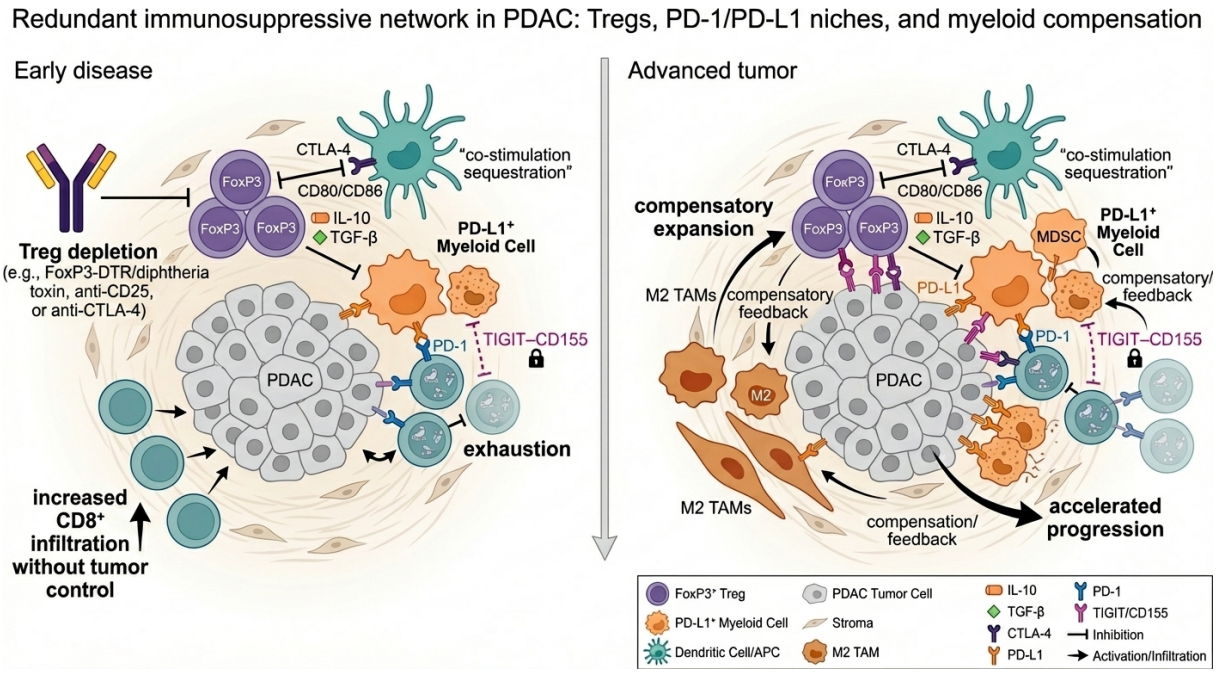

Figure 2. Redundant, stage-dependent immunosuppression in PDAC. *Two panels contrast early disease (left) with advanced tumor (right). FoxP3+ regulatory T cells (Tregs) suppress anti-tumor immunity by secreting IL-10 and TGF-β and by sequestering co-stimulation through CTLA-4–mediated removal of CD80/CD86 from dendritic cells. In parallel, PD-L1+ myeloid cells engage PD-1 on CD8+ T cells to drive exhaustion, and the TIGIT–CD155 axis provides a parallel, redundant checkpoint (Freed-Pastor et al., 2021). Experimental Treg depletion (e.g., FoxP3-DTR/diphtheria toxin, or anti-CD25; anti-CTLA-4 also depletes intratumoral Tregs) increases CD8+ T-cell infiltration but, per Zhang et al. (2020), does not yield tumor control — at the precursor/early stage it accelerates carcinogenesis through loss of tumor-restraining myofibroblastic CAFs. In advanced tumors, removing one suppressive node triggers compensatory expansion of MDSCs and M2-polarized TAMs and reinforcement of PD-L1 and TIGIT–CD155 signaling, accelerating progression. The figure emphasizes that single-axis immunosuppression blockade is buffered by redundant myeloid and checkpoint compensation.*

While the stromal and cellular barriers described above provide a formidable extrinsic defense, PDAC tumor cells further reinforce this evasion through profound cell-intrinsic mechanisms (Figure 3). Pancreatic tumors exhibit an inherently “cold” immunogenic profile, generating few recognizable neoantigens to alert the immune system. Genomic analyses show that PDAC has among the lowest tumor mutational burdens (TMB) of any major cancer—on the order of only a few dozen nonsynonymous mutations per tumor, far fewer than immunogenic malignancies such as melanoma (Waddell et al., 2015; Bailey et al., 2016). This paucity of neoantigens translates into weak T-cell recognition of PDAC as “non-self.” Indeed, immunogenomic studies indicate that most PDACs evolve with little evidence of immunoediting by T cells (Balachandran et al., 2017; ■uksza et al., 2022). Consistently, only rare PDAC cases with hypermutation (~1% of patients) generate robust spontaneous T-cell responses or show clinical benefit from immune checkpoint blockade (Balachandran et al., 2017; Lawlor et al., 2021). Identifying which specific antigen classes actually drive protective T-cell responses, particularly in rare long-term survivors, remains a major area of active investigation.

Beyond lacking targets for immune recognition, PDAC cells actively avoid antigen presentation by downregulating the machinery required for T-cell detection. A notable proportion of pancreatic cancers have deficient surface expression of major histocompatibility complex class I (MHC-I), which cripples their visibility to cytotoxic CD8^+ T lymphocytes. Unlike melanoma, where immune escape often involves hardwired genetic mutations in HLA genes or beta-microglobulin (B2M), PDAC rarely harbors such permanent lesions (Yamamoto et al., 2020). Instead, pancreatic tumor cells employ reversible mechanisms to suppress MHC-I. Recent mechanistic work showed that

PDAC cells sequester and degrade MHC-I molecules in lysosomes via an autophagy-dependent process, leading to profoundly reduced MHC-I at the cell surface despite intact genes (Yamamoto et al., 2020). This loss of antigen presentation creates an immune “blind spot,” allowing cancer cells to proliferate unnoticed. These tumor-intrinsic MHC-I deficits are often compounded by the extrinsic immunosuppressive signals previously discussed, such as PD-L1 upregulation and the secretion of TGF-\beta and IL-10 by the stroma (Feig et al., 2013; Bellone et al., 1999).

A third tumor-intrinsic barrier is a pronounced deficit in functional antigen-presenting cells—specifically, the Batf3-dependent type 1 conventional dendritic cells (cDC1) needed to prime CD8^+ T-cell responses. In pancreatic cancer, these cross-priming cDC1 cells are exceptionally scarce or functionally impaired (Hegde et al., 2020). Both murine and clinical studies have found that PDAC tumors tend to exclude cDC1, often confining them to small peritumoral tertiary lymphoid aggregates (Burrack et al., 2022). This paucity is traced to tumor-driven disruption of dendritic cell recruitment; PDAC cells secrete factors (M-CSF, GM-CSF, G-CSF) that preferentially expand suppressive monocytes while interfering with DC development (Burrack et al., 2022). Restoration of cDC1 activity can reverse this bottleneck: administration of Flt3L in a KPC mouse model expanded intratumoral cDC1 numbers, reinvigorating local CD8^+ T cells and slowing tumor progression (Hegde et al., 2020). Conversely, in the absence of Batf3-dependent cDC1, anti-tumor CD8^+ T cells remain poorly primed and ineffective even when given potent immunotherapies (Burrack et al., 2022).

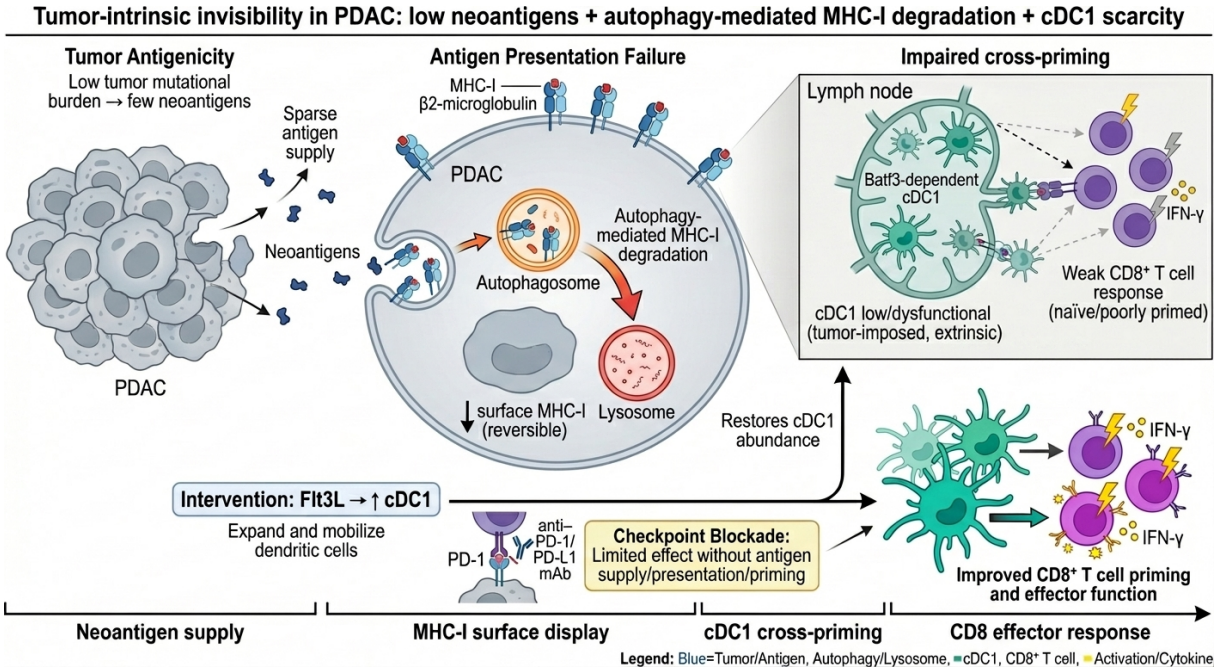

Figure 3. Cell-intrinsic and tumor-imposed barriers to CD8+ T-cell recognition in PDAC, *read left to right along the antigen pathway. (1) Antigenicity: PDAC's low tumor mutational burden yields few neoantigens and a sparse antigen supply (Waddell et al., 2015; Bailey et al., 2016). (2) Antigen presentation: surface MHC-I (heavy chain plus β2-microglobulin) is reduced not by genetic loss but by NBR1-dependent selective autophagy that routes MHC-I to lysosomes for degradation — a reversible process restorable by autophagy inhibition (Yamamoto et al., 2020). (3) Cross-priming: Batf3-dependent type 1 conventional dendritic cells (cDC1) — host cells whose numbers are suppressed by tumor-derived factors — are scarce or dysfunctional in tumor-draining lymph nodes, creating a priming bottleneck so that CD8+ T cells remain naïve/poorly primed (Hegde et al., 2020; Burrack et al., 2022). (4) Effector response: without adequate antigen supply, surface MHC-I, and cDC1-mediated priming, checkpoint blockade (anti–PD-1/PD-L1) has limited effect. Therapeutic restoration with Flt3L expands and mobilizes cDC1, relieving the bottleneck and improving CD8+ T-cell priming and IFN-γ effector function — most effectively when combined with measures that restore antigen supply and MHC-I display.*

Metabolic factors further compound this immune privilege, erecting multiple biochemical barriers that act in concert with the dense desmoplastic stroma and immunosuppressive cellular networks (Figure 4). In PDAC, a **hypoxic** and nutrient-deprived stroma drives the accumulation of extracellular **adenosine**, creating a metabolic barrier to immune attack (Liu et al., 2025). Tumor cells and CAFs express high levels of the ecto-enzymes **CD39** and **CD73**, which sequentially hydrolyze ATP to adenosine in the TME (Deaglio et al., 2007; Zhao et al., 2021). The resulting adenosine primarily engages high-affinity A2A adenosine receptors on effector T and NK cells, triggering intracellular cAMP signaling that blunts their cytotoxic functions; at higher concentrations, adenosine also activates low-affinity A2B receptors on myeloid and stromal cells, promoting immunosuppressive gene expression and angiogenesis (Ohta et al., 2006; Faraoni et al., 2023). Clinically, elevated CD73 expression in PDAC correlates with an exclusion of effector T cells from tumor islets and with worse patient survival outcomes (Zhao et al., 2021; Faraoni et al., 2023). Adenosine signaling further amplifies regulatory pathways: FoxP3^+ Tregs respond to adenosine by upregulating immunosuppressive programs via A2A receptors, and Tregs themselves contribute to adenosine production through CD39/CD73, establishing a self-reinforcing suppressive loop (Deaglio et al., 2007; Liu et al., 2025). Consistent with these mechanisms, experimental inhibition of CD73 in murine PDAC models unleashes anti-tumor immunity by reducing MDSC accumulation and restoring intratumoral T-cell activation (King et al., 2022). However, an important unanswered question concerns which cellular compartment serves as the dominant *in situ* source of CD39/CD73 activity and how spatially restricted A2A versus A2B signaling is across distinct hypoxic–acidic niches.

Beyond purinergic signaling, PDAC tumors deploy **amino acid-depleting enzymes** to starve effector lymphocytes and skew the immune balance. **Indoleamine 2,3-dioxygenase 1 (IDO1)** is often upregulated in PDAC and antigen-presenting cells in response to IFN-\gamma, leading to rapid catabolism of local tryptophan pools into kynurenine (Uyttenhove et al., 2003; Anu et al., 2023). T cells are exquisitely sensitive to tryptophan depletion: low tryptophan activates the stress kinase GCN2, causing cell-cycle arrest and anergy, while kynurenine engages aryl hydrocarbon receptors on naïve CD4^+ T cells to drive their differentiation into FoxP3^+ Tregs (Anu et al., 2023). In parallel, infiltrating myeloid cells—particularly M2-polarized TAMs and granulocytic MDSCs—express high levels of **arginase-1 (Arg1)**, which depletes extracellular L-arginine (Bronte et al., 2003; Menjivar et al., 2023). L-arginine is critical for T-cell receptor \zeta-chain expression; its removal effectively paralyzes cytotoxic T cells and NK cells by impairing TCR signaling and metabolic fitness (Rodriguez et al., 2002). Consistent with this mechanism, Arg1^+ immunosuppressive macrophages expand as PDAC progresses and are associated with poor CD8^+ T-cell infiltration in patient tumors (Menjivar et al., 2023). Notably, in PDAC mouse models, genetic ablation or pharmacologic inhibition of Arg1 in macrophages can alleviate immune suppression, although tumors often compensate via alternative pathways (Menjivar et al., 2023).

Chronic hypoxia is a defining feature of PDAC that exacerbates these immunosuppressive metabolic pathways. Poor vascularization and dense fibrosis stabilize HIF-1\alpha, shunting cellular metabolism toward anaerobic glycolysis and the production of **lactic acid**, which acidifies the tumor interstitium (Yuen & Díaz, 2014); measured interstitial pH in such tumors falls to ~6.5–6.8 (Gottfried et al., 2006). Such an **acidic microenvironment** directly undermines immune cell function: tumor-infiltrating T cells exposed to low pH show reduced proliferation and IFN-\gamma/TNF-\alpha secretion (Fischer et al., 2007). Mechanistically, acidosis can restrict T-cell metabolic programs, preserving a less differentiated, stem-like state at the expense of immediate effector function (Cheng et al., 2023). Dendritic cell biology is likewise disrupted, as DCs exhibit impaired antigen presentation and instead skew toward a tolerogenic phenotype (Gottfried et al., 2006). Moreover, lactic acid serves as a signaling metabolite that conditions macrophages toward an M2-like

phenotype characterized by Arg1 upregulation and high IL-10 production (Colegio et al., 2014). In PDAC, lactate accumulation and associated protein lactylation promote an abundance of protumoral macrophages and a dearth of cytotoxic T cells in hypoxic regions, contributing to a "cold" tumor architecture (Sun et al., 2025). This raises the question of whether lactate-driven macrophage “training” is truly causative for immune exclusion or if it serves mainly as a correlate of the broader hypoxic state.

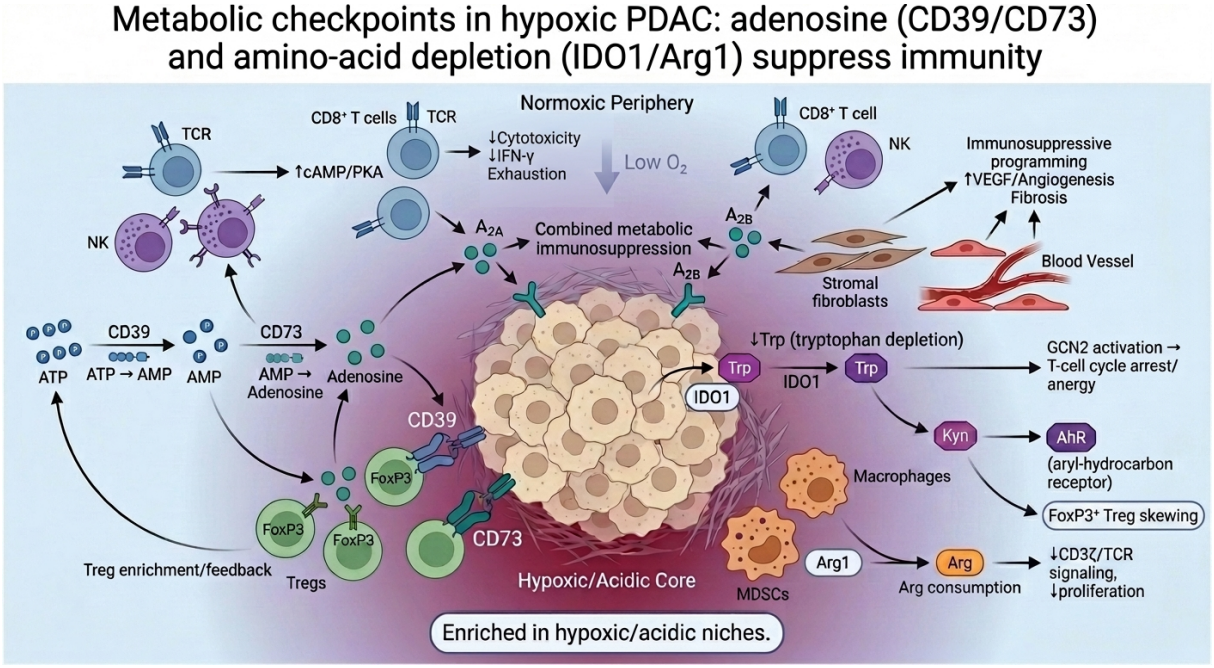

Figure 4. Interlocking metabolic checkpoints that suppress immunity in the hypoxic, acidic PDAC core. *Adenosine arm (left): tumor cells, CAFs, and Tregs co-express the ecto-enzymes CD39 (ATP→AMP) and CD73 (AMP→adenosine), generating extracellular adenosine enriched in hypoxic niches (Deaglio et al., 2007; Zhao et al., 2021). Adenosine engages high-affinity A2A receptors on CD8+ T and NK cells, raising cAMP/PKA and blunting cytotoxicity and IFN-γ (Ohta et al., 2006); at higher concentrations it engages low-affinity A2B receptors on myeloid, stromal, and endothelial cells, promoting immunosuppressive programming, VEGF-driven angiogenesis, and fibrosis (Faraoni et al., 2023). FoxP3+ Tregs both respond to and amplify this axis (CD39/CD73 feedback). Amino-acid arm (right): IDO1 (induced by IFN-γ) catabolizes tryptophan to kynurenine. Two mechanistically distinct consequences follow: local tryptophan depletion activates the stress kinase GCN2 in T cells, causing cell-cycle arrest and anergy; and kynurenine engages the aryl-hydrocarbon receptor (AhR) to skew naïve CD4+ T cells toward FoxP3+ Tregs (Uyttenhove et al., 2003; Anu et al., 2023). In parallel, M2-like TAMs and granulocytic MDSCs express arginase-1 (Arg1), depleting L-arginine and downregulating the TCR CD3ζ chain, impairing T-cell proliferation and NK function (Rodriguez et al., 2002; Menjivar et al., 2023). Chronic hypoxia and low pH amplify all of these programs. The arms interlock (A2B-recruited myeloid cells supply Arg1; kynurenine-induced Tregs reinforce CD39/CD73), producing redundant metabolic immunosuppression concentrated in hypoxic/acidic niches.*

Breaking these metabolic barriers has proven challenging due to the redundant and interlinked nature of these pathways. For instance, adenosine signaling via A2B receptors promotes the recruitment of myeloid cells that supply Arg1, while IDO1-generated kynurenine fosters Tregs that augment adenosine production via CD39/CD73 (Deaglio et al., 2007; King et al., 2022). Consequently, therapeutic strategies are now exploring combined metabolic checkpoint blockade. Early-phase trials have tested antibodies against CD73 (e.g., oleclumab) and A2A receptor antagonists in PDAC patients, typically with anti-PD-1/PD-L1 inhibitors, although clinical benefits have so far been limited (Zhao et al., 2021). IDO1 inhibitors (e.g., epacadostat) failed to improve outcomes in phase III melanoma trials, casting doubt on single-agent efficacy (Long et al., 2019). Similarly, arginase inhibition (e.g., CB-1158) can reverse myeloid-mediated suppression in preclinical settings (Steggerda et al., 2017) but has not yet demonstrated clear success in PDAC patients. Future trials may need to couple metabolic blockade with stromal-targeted agents like

CXCL12 or TGF-\beta inhibitors to meaningfully restore immune surveillance.

3. Preclinical PDAC Models: GEMMs, Syngeneic Transplants, and Humanized Systems

Over the past decade, recognizing these multifaceted immune barriers has prompted an intensive search for strategies to invigorate anti-tumor immunity in PDAC. A cornerstone of this effort has been the use of **preclinical PDAC models**—especially immunocompetent mouse models—to dissect immune evasion mechanisms and to test novel therapeutic approaches in contexts that closely mimic human disease.

3.1 Genetically engineered mouse models (GEMMs): spontaneous PDAC with desmoplasia and immune editing

Genetically engineered mouse models (GEMMs) of pancreatic cancer have been pivotal for investigating immune mechanisms in an autochthonous tumor setting. The prototypical GEMM is the KPC model, which carries conditional mutations in *Kras* (G12D) and *Trp53* (R172H) under a pancreas-specific Cre recombinase, leading to spontaneous PDAC development in immunocompetent mice (Hingorani et al., 2005). KPC mice recapitulate the defining histopathology of human PDAC, notably a pronounced desmoplastic stroma and sparse tumor-infiltrating lymphocytes. The progressing tumors are encased in a dense extracellular matrix produced by CAFs, and immunohistochemical analyses show an abundance of macrophages and granulocytic myeloid cells but a striking paucity of CD8^+ T cells within tumor islets (Metzger et al., 2019). This mirrors the immune-excluded phenotype of human pancreatic tumors and provides a faithful model of the profoundly immunosuppressive TME characteristic of PDAC (Pham et al., 2021). Consistent with clinical observations, autochthonous KPC tumors are largely unresponsive to checkpoint inhibitor monotherapy, as they lack the pre-existing T cell infiltrates needed for anti–PD-1 or anti–CTLA-4 antibodies to be effective (Feig et al., 2013).

Importantly, GEMMs enable the study of **cancer immunoediting** during PDAC evolution. In KPC mice, neoplastic lesions emerge and progress in the continuous presence of a functional immune system, allowing for potential immune “sculpting” of the tumor antigen landscape. In practice, PDAC’s notoriously low antigenicity limits the extent of immunoediting: KPC tumors generally harbor an extremely low neoantigen burden, and their growth often proceeds unchecked by adaptive immunity (Evans et al., 2016). A seminal study demonstrated that spontaneous KPC tumors developed at comparable rates in immunocompetent mice and in T cell–deficient hosts, indicating an absence of effective immune elimination of emerging cancer cells (Evans et al., 2016). Indeed, no dominant cytotoxic T-cell response arises against KPC tumors because these tumors present few recognizable neoepitopes and can downregulate antigen-presentation machinery early in their development. However, the principle of immunoediting was affirmed when KPC tumor cells were engineered to express a strong model neoantigen (ovalbumin): under this enforced immune pressure, tumors were rejected and long-term T cell memory was established, whereas the same antigen-expressing tumors escaped immune control in T cell–tolerant hosts (Evans et al., 2016). These findings underscore that KPC mice can reveal whether PDAC progression is constrained by immunity or simply invisible to it.

Despite their high fidelity to human disease, PDAC GEMMs have notable limitations. One challenge is the long and variable latency of tumor development; KPC mice typically require 3–6 months to develop overt pancreatic tumors, with a median survival of around 5 months (Hingorani et al., 2005). This protracted timeline makes large-scale therapeutic studies laborious. Moreover, because GEMM tumors arise from a defined set of engineered driver mutations, their genomic diversity is limited compared to sporadic human PDAC. KPC tumors lack the breadth of subclonal heterogeneity found

in advanced human tumors, and their spectrum of neoantigens is even narrower (Pham et al., 2021).

3.2 Syngeneic and orthotopic PDAC models: immunocompetent platforms for therapy testing

While GEMMs provide a spontaneous developmental context, syngeneic transplantable models offer a more flexible platform for rapid therapeutic testing. Unlike patient■derived xenografts that require immunodeficient hosts, syngeneic mouse models of PDAC involve implanting murine pancreatic cancer cells into genetically matched, immunocompetent mice—thereby preserving an intact host immune system and physiologic tumor–immune interactions in vivo (Pham et al., 2021; Ju et al., 2024).

The implantation site profoundly influences the tumor immune microenvironment in these models. Tumor cells can be introduced at a **heterotopic** site (e.g., subcutaneous flank) or **orthotopically** in the native pancreas; importantly, orthotopic implantation engages organ■specific stromal and immune components that are absent in simple subcutaneous grafts (Pham et al., 2021; Wang et al., 2022). Orthotopic pancreatic tumors elicit a far more pronounced fibroinflammatory response, developing significantly greater collagen deposition (desmoplasia) and higher infiltration of immunosuppressive myeloid cells (e.g., Gr1^+ MDSCs and F4/80^+ macrophages) than genetically identical tumors grown subcutaneously (Wang et al., 2022; Pham et al., 2021). These findings indicate that orthotopic tumors engage tissue-specific factors—such as pancreatic stellate cells and tissue-resident macrophages—that shape an immune-exclusionary microenvironment closely resembling human PDAC (Wang et al., 2022). Consequently, immune checkpoint blockade that might substantially slow the growth of a subcutaneous tumor often shows minimal efficacy against an orthotopic pancreatic tumor, mirroring the clinical resistance of PDAC to immunotherapy (Wang et al., 2022).

Multiple murine PDAC cell lines are used in these platforms, each with distinct tumor genetics and immunogenicity. The **Panc02 (Pan02)** cell line—derived from a chemically induced tumor—is one of the most established models (Pham et al., 2021). However, Pan02 tumors are notable for an *exceptionally high mutational burden and neoantigen load relative to typical human PDAC*, with a high burden of nonsynonymous mutations (Kinkead et al., 2018). This hypermutated status stems from its origin as a carcinogen-induced tumor, and Pan02 has been reported to **lack the canonical oncogenic drivers** (e.g., **KRAS^G12D**) ubiquitous in human PDAC (Pham et al., 2021). Due to its abundant neoantigens, the Pan02 model retains an inherent *immunogenicity* that can be unmasked by therapies; multiple studies have reported robust responses to checkpoint inhibitors in subcutaneous Pan02 tumors (Sandin et al., 2014; Mace et al., 2018). This stands in stark contrast to **clinical PDAC**, where single-agent checkpoint inhibitors have elicited minimal response rates (O'Reilly et al., 2019). The discrepancy suggests that Pan02's high neoantigen burden artificially sensitizes it to immunotherapy, potentially **exaggerating the apparent efficacy of checkpoint blockade** (Kinkead et al., 2018; Ju et al., 2024).

To better emulate human PDAC, researchers have developed **syngeneic lines derived from GEMMs**, such as the **KPC-derived lines** (Pham et al., 2021). These tumors recapitulate many features of patient tumors, including a **dense collagenous stroma and an immunosuppressive leukocyte infiltrate** dominated by M2 macrophages and Tregs (Pham et al., 2021). KPC-derived tumors appear to maintain an immune-excluded phenotype regardless of implantation site and, like human PDAC, are **poorly immunogenic and unresponsive to single-agent checkpoint inhibitors** (Pham et al., 2021; O'Reilly et al., 2019). This divergence was highlighted by Pan et al. (2019), who found that combining anti-CD47 with PD-L1 blockade yielded synergistic inhibition in a Panc02 model but **had no effect in a second, less immunogenic PDAC model** (Pan et al., 2019).

As discussed in Section 4, overcoming this resistance in orthotopic or KPC-derived models typically requires multi-modal priming strategies to convert these "cold" tumors into "hot" ones (Ju et al., 2024).

3.3 Humanized and patient-derived xenograft (PDX) models: bridging to human immunity

To address the translational gaps inherent in purely murine systems, researchers have developed humanized mouse models and patient-derived xenografts (PDXs) that incorporate human immune components. In humanized PDAC models, severely immunodeficient mice (e.g., NOD/SCID-IL2r\gamma^null strains) are engrafted with human hematopoietic stem cells (CD34^+ HSCs) or peripheral blood mononuclear cells (PBMCs), enabling partial reconstitution of a human immune system in vivo (Walsh et al., 2017; He et al., 2020). Advanced humanized mouse strains now incorporate human cytokine genes (e.g., M-CSF, IL-3/GM-CSF, IL-6) to improve human myeloid cell and T-cell development (Walsh et al., 2017; De La Rochere et al., 2018). These humanized mice can be implanted with human PDAC cells or tissues, creating chimeric models where human immune cells interact with the human TME (He et al., 2020). For example, orthotopic humanized PDAC models have demonstrated dense, human-like desmoplastic stroma formation and measurable infiltration of human T-cell and myeloid subsets, providing a platform to evaluate human-specific immunotherapies—such as anti-PD-1/PD-L1 antibodies or engineered T-cell therapies—that require human targets absent in conventional murine models (Jeong et al., 2023).

Patient-derived xenograft (PDX) models of PDAC involve direct implantation of a patient's tumor tissue into immunodeficient mice, maintaining the authentic genetic and histopathological characteristics of the original tumor (Sereti et al., 2018). These models retain the intratumoral heterogeneity and clonal architecture of human tumors better than cell line models and have shown a higher correlation with clinical treatment outcomes (Aparicio et al., 2015). To evaluate immune-based therapies in PDX models, researchers have introduced human immune cells into PDX-bearing mice, creating humanized PDX platforms (De La Rochere et al., 2018). Reconstituted with autologous or HLA-matched immune cells, these platforms serve as individualized "avatars" to test personalized immunotherapies, such as neoantigen-targeted T cells or vaccines, against a patient's own tumor in vivo (Sereti et al., 2018). For instance, adoptive transfer of a patient's own expanded tumor-infiltrating lymphocytes (TILs) could mediate regression of the corresponding PDAC PDX tumor in an IL-2–supplemented humanized mouse (Nilsson et al., 2022).

Notably, humanized PDAC models have facilitated preclinical trials of novel immunotherapies that cannot be evaluated in syngeneic mouse systems. Chimeric antigen receptor (CAR) T cells targeting human tumor antigens, such as the PDAC-associated mucin MUC1, have been studied in NSG mice engrafted with human PDAC tumors and human immune effectors (Posey et al., 2016). These studies showed that human CAR T cells could traffic to and control the growth of pancreatic tumor xenografts, while also revealing disparities in toxicity profiles between mice and humans (Posey et al., 2016). Similarly, humanized models have been used to assess adoptive NK cell transfers, allowing investigators to monitor human immune cell activation and cytokine release in a setting that approximates human biology (He et al., 2020).

Despite their promise, humanized and PDX models of PDAC come with significant limitations. Human immune reconstitution is inherently incomplete; key components like functional lymph node architecture and a human-specific cytokine network are only partially realized (Walsh et al., 2017). Furthermore, unless the human immune cells and tumor implant are autologous, allo-reactivity can occur—introduced human T cells may recognize the engrafted tumor as "foreign" due to HLA mismatches, leading to artifactual T-cell infiltration (Walsh et al., 2017; He et al., 2020). On the tumor side, conventional PDX models lack the patient's supportive stroma; human fibroblasts are

progressively replaced by murine stromal elements as the xenograft grows (Delitto et al., 2015). Consequently, the fibrotic matrix in orthotopic humanized PDAC xenografts is largely generated by mouse fibroblasts, which may not faithfully recapitulate the heterogeneity of human CAF subtypes (Jeong et al., 2023; Sereti et al., 2018).

4. Immune-Based Therapeutic Strategies in Preclinical PDAC Models

Building upon the mechanistic insights provided by these diverse preclinical platforms, researchers have sought to develop strategies that can overcome the inherent resistance of PDAC to immunotherapy.

4.1 Checkpoint blockade in PDAC models: efficacy limits and combination approaches

As established in the preceding sections, checkpoint blockade alone has consistently failed to induce meaningful anti-tumor effects in pancreatic cancer models. In immunocompetent murine PDAC systems, such as the genetically engineered KPC model, single-agent PD-1 or CTLA-4 checkpoint inhibitors produce negligible tumor regression and no survival benefit (Winograd et al., 2015; Vonderheide, 2018). This refractoriness is largely attributed to PDAC's "immune desert" microenvironment: baseline effector T-cell infiltration is extremely low, tumor epithelium remains segregated by dense desmoplastic stroma, and immunosuppressive myeloid cells dominate the tumor milieu (Winograd et al., 2015). Even though PD-L1 is detectable on PDAC cells and stromal macrophages, the lack of pre-existing cytotoxic T cells means there are few targets for checkpoint blockade to "release" (Winograd et al., 2015).

Effective anti-tumor immunity in PDAC models emerges only when checkpoint blockade is combined with therapies that recruit or activate T cells. Robust tumor control has required multi-component immunotherapy strategies to first initiate a T-cell response against the tumor. The most striking preclinical successes have come from coupling checkpoint inhibitors with a priming agent such as a vaccine or co-stimulatory agonist that generates new tumor-specific T cells. One approach introduced a tumor cell vaccine to "seed" the tumor with primed T cells. In a landmark experiment, vaccinating KPC mice with GM-CSF–secreting allogeneic pancreatic tumor cells (GVAX) induced notable T-cell infiltration into previously barren tumors; only under those conditions did subsequent PD-1 plus CTLA-4 blockade slow tumor progression and improve survival (Winograd et al., 2015). Similarly, providing a potent co-stimulatory signal through OX40 (CD134) alongside PD-1 blockade converted previously unresponsive tumors into responsive ones. In one study, the combination of PD-1 inhibition with an agonistic OX40 antibody eradicated established PDAC tumors and generated long-term immune memory in mice by reprogramming intratumoral T cells from an exhausted phenotype toward an active, interferon-producing effector phenotype (Ma et al., 2020).

Therapies that activate dendritic cells and innate immunity can further synergize with checkpoint blockade to overcome PDAC's "cold" microenvironment. Agonists of co-stimulatory receptors on antigen-presenting cells (such as CD40) and activators of innate immune pathways (such as STING agonists) have emerged as effective partners to PD-1/PD-L1 blockade. Engaging CD40 on dendritic cells and macrophages provides a surrogate "licensing" signal that bypasses the need for T-helper lymphocytes, in turn promoting antigen presentation and cross-priming of T cells in the tumor microenvironment (Vonderheide, 2018). In an orthotopic KPC transplant model, treatment with an agonistic CD40 antibody markedly altered the immune landscape: formerly excluded CD8^+ T cells infiltrated into tumor islets, macrophages shifted toward a pro-inflammatory state, and dendritic cells became more activated (Ichikawa et al., 2026). Ichikawa and colleagues demonstrated that neither dual checkpoint blockade nor CD40 agonist

alone was sufficient to induce tumor regression, but the combination elicited robust anti-tumor immunity and a significant reduction in tumor burden (Ichikawa et al., 2026). Similarly, pharmacologic activation of the STING pathway can act as an *in situ* vaccine. In a recent study, administering a potent cyclic dinucleotide STING agonist to KPC tumor–bearing mice provoked an influx of innate immune cells and robust cross-priming of CD8^+ T cells; when combined with checkpoint blockade, this strategy reversed the immune privilege of pancreatic tumors and led to markedly enhanced T cell–dependent tumor regression (Ager et al., 2021).

4.2 Targeting immunosuppressive myeloid cells in PDAC: CSF1R inhibitors and CD40 agonists

While innate immune activation via CD40 or STING agonists represents one avenue for converting “cold” tumors into T-cell–inflamed lesions, a parallel strategy focuses on directly dismantling or reprogramming the broader immunosuppressive myeloid network that enforces this state. One strategy to counter PDAC’s immunosuppressive myeloid compartment has been to deplete tumor-associated macrophages (TAMs) via CSF1–CSF1R blockade. In murine PDAC models, treatment with a CSF1R inhibitor (e.g., the small-molecule PLX3397) drastically reduced TAM numbers, which in turn relieved local T-cell suppression and facilitated CD8^+ T-cell infiltration into the TME (Zhu et al., 2014). Zhu et al. (2014) demonstrated that CSF1R blockade not only slowed primary tumor growth but also “re-educated” the remaining macrophages toward a more pro-inflammatory phenotype, creating conditions more permissive for cytotoxic T cells to control the cancer. Notably, combining CSF1R inhibition with T-cell checkpoint blockade yielded synergistic anti-tumor effects in these models—an outcome attributed to the unleashing of T-cell activity once TAM-mediated immunosuppression was lifted (Zhu et al., 2014).

As introduced in the context of dendritic cell licensing, agonistic CD40 antibodies also exemplify the strategy of functional reprogramming. In KPC mouse models, a CD40 agonist induced potent cytotoxic T-cell responses and even tumor regression, despite baseline resistance to checkpoint inhibitors (Beatty et al., 2011). Beatty and colleagues demonstrated that CD40-stimulated macrophages can directly attack the tumor stroma and facilitate T-cell infiltration, effectively converting “T-cell–cold” PDAC tumors into inflamed, T-cell–rich lesions. Building on this, Winograd et al. (2015) reported that combining CD40 activation with checkpoint blockade (or with chemotherapy) overcame PD-1/CTLA-4 resistance in PDAC models, leading to significantly improved survival.

In parallel, blocking the recruitment of immunosuppressive myeloid cells is another effective tactic in preclinical PDAC studies. Intercepting pathways like CCL2/CCR2 or CXCL1/2/CXCR2 curtailed the influx of monocytes and neutrophils, resulting in reduced TAM and MDSC accumulation and enhanced anti-tumor T-cell activity (Nywening et al., 2018; Steele et al., 2016). In the study by Steele et al. (2016), CXCR2 blockade not only slowed metastasis but also augmented the efficacy of anti–PD-1 therapy, indicating that neutrophils actively contribute to immunotherapy resistance. Intriguingly, combined blockade of CCR2^+ monocytes and CXCR2^+ neutrophils yielded additive benefits, improving responses to chemotherapy and checkpoint inhibition in pancreatic tumor models (Nywening et al., 2018). Collectively, these preclinical insights highlight that dismantling the immunosuppressive myeloid network—whether by depletion, functional reprogramming, or trafficking blockade—can substantially restore anti-tumor immunity in pancreatic cancer.

4.3 Adoptive cell therapies in PDAC models: CAR T cells and TCR-engineered T cells

As an alternative to activating endogenous T cells, **adoptive cellular therapies** are being actively explored in preclinical PDAC models, using genetically engineered T cells to bypass the tumor’s inherent recognition deficits. CAR T cells directed against PDAC-associated antigens, notably

mesothelin, can recognize and lyse pancreatic tumor cells in vitro and in mouse models (Beatty et al., 2014). However, early studies showed that CAR T-cell monotherapy yields only limited tumor regression in vivo, owing to the formidable immunosuppressive microenvironment of PDAC that curtails T-cell infiltration and persistence (Beatty et al., 2014; Feig et al., 2013).

To improve CAR T-cell efficacy, recent preclinical work has focused on modifying either the T cells themselves or the tumor stroma to enable better infiltration. Emerging directions include the development of CAR T cells armed with enzymes like **heparanase** to digest the extracellular matrix (Caruana et al., 2015) or bioengineered to secrete bispecific T-cell engagers against fibroblast activation protein (FAP) (Wehrli et al., 2024). Other “armored” enhancements include the local release of immunostimulatory cytokines, such as IL-12, which has been shown to repolarize the suppressive microenvironment and improve T-cell persistence (Zhu et al., 2024).

In parallel, TCR-engineered T cells provide a strategy to target tumor antigens not accessible on the cell surface, most notably neoantigens arising from driver gene mutations. Given that **~90%** of PDAC tumors harbor activating *KRAS* mutations, mutant KRAS represents a compelling target. Preclinical studies confirm that TCR-transduced T cells can selectively kill pancreatic cancer cells expressing target mutant peptides, such as KRAS^{G12D} or KRAS^{G12V} (Lu et al., 2023; Bear et al., 2024). Encouragingly, the KRAS-directed TCR approach has transitioned from preclinical validation to clinical proof-of-concept. Early evidence from Tran et al. (2016) showed that autologous T cells enriched for KRAS^{G12D}-specific TCRs could mediate regression of metastatic lesions. This translational momentum culminated in a recent first-in-human study demonstrating that TCR gene therapy can induce objective responses in advanced PDAC (Leidner et al., 2022). As discussed in Section 5.3, while these results confirm the therapeutic potential of targeting driver mutations, they also highlight critical mechanisms of adaptive resistance that must be addressed in future designs.

4.4 Cancer vaccines and oncolytic virotherapy in PDAC preclinical studies

While adoptive cellular therapies provide external effectors, therapeutic vaccination and oncolytic virotherapy aim to ignite the endogenous immune system. A leading strategy is the GM-CSF-secreting whole-cell vaccine (GVAX), composed of irradiated pancreatic tumor cells engineered to release granulocyte-macrophage colony-stimulating factor to attract and mature dendritic cells (Soares et al., 2015). In murine PDAC models, GVAX alone can induce infiltration of CD8^+ T cells and even tertiary lymphoid structures (Lutz et al., 2014). However, this vaccine-driven immune priming also triggers adaptive immune resistance, specifically the upregulation of PD-L1 (Lutz et al., 2014; Soares et al., 2015). Co-administering GVAX with anti–PD-1 or anti–PD-L1 antibodies has yielded superior anti-tumor effects by sustaining T-cell activity (Soares et al., 2015).

Oncolytic viruses (OVs) represent a parallel approach, leveraging direct tumor cell lysis to spark an anti-cancer immune response. A variety of OVs—including engineered adenovirus, vaccinia, and measles virus—have been tested against pancreatic tumors for their ability to selectively infect KRAS-mutant or immune-evasive cancer cells (Thoidingjam et al., 2024). Upon infecting PDAC cells, OVs replicate and lyse the tumor, releasing a burst of tumor antigens and danger-associated molecular patterns (DAMPs) that activate local dendritic cells and macrophages (Guo et al., 2017). Recent preclinical advances have further enhanced OVs as dual immunotherapy agents by "arming" them with immunomodulatory genes like GM-CSF and IL-21 (Xuan et al., 2024) or enzymes like hyaluronidase to degrade the tumor extracellular matrix (Bazan-Peregrino et al., 2021).

5. Clinical Immunotherapy Trials in PDAC

While preclinical models suggest that multi-modal combinations can ignite anti-tumor immunity, the translation of these strategies into clinical success has faced significant hurdles, reflecting the

resilient and redundant nature of PDAC's immune evasion.

5.1 Checkpoint inhibitor therapy ± chemotherapy: minimal efficacy in PDAC

Immune checkpoint inhibition has thus far shown only minimal efficacy in PDAC clinical trials. In contrast to immunogenic tumors like melanoma, objective response rates to anti–PD-1 or anti–PD-L1 monotherapy in unselected, microsatellite-stable PDAC patients are typically under 5% (Al-Khinji et al., 2025). The principal exception is the rare subset of PDAC characterized by high microsatellite instability (MSI-H) or mismatch-repair deficiency. In the phase II KEYNOTE-158 study, a small MSI-H PDAC subgroup showed some objective responses to pembrolizumab, though the pancreatic-specific response rate was modest (<20%) and lower than for most other tumor types (Marabelle et al., 2020). A recent multi-center analysis of 31 patients with MSI-H metastatic PDAC reported an objective response rate of 48% and a median progression-free survival of ~27 months, underscoring the unique sensitivity of this subtype (Taieb et al., 2023).

Outside of MSI-H disease, however, immunotherapy responses in PDAC remain exceedingly uncommon. Only isolated case reports describe **"exceptional responders"** among microsatellite-stable PDAC patients—often in tumors with unusually high mutation burdens or DNA-damage repair defects—and even these anecdotal responses are rare (Sugumar et al., 2025). Early clinical trials of CTLA-4 blockade highlighted this inherent resistance: a Phase II study of ipilimumab in advanced PDAC found no objective responses or survival benefit (Royal et al., 2010). Even attempts to combine CTLA-4 and PD-1 blockade, such as durvalumab plus tremelimumab, showed no significant clinical activity in metastatic PDAC (O'Reilly et al., 2019).

Given the lack of success with monotherapies, clinical strategies have moved toward combining checkpoint inhibitors with standard chemotherapy. The rationale is that cytotoxic chemotherapy could potentiate immunotherapy by increasing tumor antigen release and modulating immunoregulatory cells (Zhang et al., 2022). However, these immunologic changes have not translated into improved patient outcomes. In randomized trials, the addition of checkpoint inhibitors to first-line chemotherapy has failed to demonstrate any significant survival benefit. Notably, the phase II CCTG PA.7 trial tested gemcitabine plus nab-paclitaxel with or without durvalumab and tremelimumab and found no prolongation of median overall survival in the immunotherapy arm (Renouf et al., 2022). Similarly, an open-label phase I study of nivolumab added to gemcitabine–nab-paclitaxel reported an objective response rate roughly in line with chemotherapy alone (Wainberg et al., 2020).

5.2 TME-modulating combination therapies in PDAC: CXCR4 inhibition, stromal depletion, and CD40 agonism

Recognizing that checkpoint blockade alone is insufficient to penetrate the PDAC architecture, clinical efforts have pivoted toward **modulating the tumor microenvironment (TME)**. As established in preclinical studies, FAP^+ fibroblasts secrete CXCL12 to physically exclude T cells (Feig et al., 2013). This rationale has moved into early-phase clinical trials using CXCR4 antagonists. For example, a Phase II study of the CXCR4 inhibitor BL-8040 plus pembrolizumab (the COMBAT trial) reported increased intratumoral CD8^+ T-cell infiltration, though clinical activity remained modest with only a ~3% partial response rate (Bockorny et al., 2020).

Another TME-targeted strategy has been **enzymatic stromal depletion** using pegylated recombinant hyaluronidase (PEGPH20). While early clinical testing suggested a potential benefit in hyaluronan-high tumors (HALO-202) (Hingorani et al., 2018), the definitive Phase III HALO-301 trial was negative, showing no improvement in overall survival and significant toxicity (Van Cutsem et al., 2020). These outcomes underscore that collapsing PDAC's physical stroma is insufficient to overcome immune suppression on its own.

A distinct approach is **agonistic CD40 immunotherapy**, which aims to hyper-activate antigen-presenting cells and reprogram macrophages. Early clinical trials confirmed episodic tumor responses: a Phase I study of agonist CD40 antibody CP-870,893 plus gemcitabine reported partial tumor regressions in \approx18% of patients (Beatty et al., 2013). More recent combination regimens, such as the agonist CD40 antibody sotigalimab with gemcitabine/nab-paclitaxel and PD-1 blockade, yielded an encouraging objective response rate in a Phase 1b trial (O'Hara et al., 2021). However, definitive survival gains have yet to be demonstrated. Finally, **radiotherapy combined with checkpoint immunotherapy** has been investigated, but results have been disappointing, with low response rates and profound treatment-related lymphopenia (Xie et al., 2020; Vošmik et al., 2024).

5.3 Therapeutic vaccines and adoptive T-cell therapies in PDAC trials

While TME modulation aims to dismantle barriers, therapeutic cancer vaccines and adoptive T-cell transfers seek to actively provide specific effector populations. Therapeutic cancer vaccines have been extensively tested, but clinical efficacy has been limited. An allogeneic GM-CSF–secreting tumor-cell vaccine (GVAX), whose mechanism for dendritic cell maturation was described in Section 4.4, showed initial promise when combined with a live-attenuated *Listeria monocytogenes* vector expressing mesothelin (CRS-207) (Le et al., 2015). However, a subsequent randomized Phase IIb/III trial (ECLIPSE) found that adding GVAX+CRS-207 to second-line chemotherapy did **not** improve survival (Le et al., 2019). Similarly, a phase III trial of algenpantucel-L failed to show any survival benefit (Hewitt et al., 2022).

A new wave of trials has explored **personalized neoantigen vaccines**. In a Phase I adjuvant trial, an individualized mRNA neoantigen vaccine (autogene cevumeran) administered after surgical resection induced de novo high-magnitude neoantigen-specific T-cell responses in half of the patients (8 of 16) (Rojas et al., 2023). Notably, those patients who mounted high-magnitude T-cell responses had markedly prolonged recurrence-free survival, suggesting that vaccine-elicited T cells can meaningfully delay PDAC progression in the adjuvant setting (Rojas et al., 2023).

Adoptive T-cell transfer is being investigated as a parallel strategy. CAR T cells targeting mesothelin were among the first tested; while safe, anti-tumor effects were limited to stable disease, illustrating how the dense stroma thwarts CAR T-cell function (Beatty et al., 2018). TCR-engineered lymphocytes targeting intracellular driver mutations have shown more dramatic, albeit transient, activity. Leidner et al. (2022) reported a patient with refractory metastatic PDAC treated with autologous T cells engineered to express a TCR specific for the KRAS^{G12D} mutation. This one-time cell infusion produced a partial response of 62% in target (lung) lesions at 1 month, deepening to 72% at 6 months, with the response ongoing at 6 months and engineered T cells still constituting >2% of circulating T cells (Leidner et al., 2022). Mechanisms of escape such as loss of the HLA allele presenting the mutant KRAS peptide have been documented in the analogous KRAS^G12D TCR setting (Tran et al., 2016) and remain a key concern for durability. This underscores the need for multi-specific products that preempt antigen or HLA loss.

6. Translational Challenges in PDAC Immunotherapy

Despite the sophisticated modeling and diverse therapeutic attempts described above, a significant disconnect remains between preclinical success and clinical reality. This translational gap is rooted in several fundamental differences between murine systems and human patients, ranging from microbial ecosystems to the temporal dynamics of tumor evolution.

6.1 Preclinical–clinical immune response gaps in PDAC: microbiome, tumor evolution, biomarkers, and safety

One major translational gap stems from differences in the tumor microbiome. Human PDAC tumors harbor a distinct intratumoral microbiome that is largely absent in laboratory mice kept under specific pathogen-free conditions. Notably, certain intratumoral bacteria in human PDAC—particularly Gammaproteobacteria—can metabolize the chemotherapeutic gemcitabine into an inactive form via bacterial cytidine deaminase, thereby conferring drug resistance in patients (Geller et al., 2017). Mouse PDAC models, which lack these tumor-colonizing bacteria, do not recapitulate this microbiome-driven chemoresistance, potentially contributing to discrepancies in therapeutic efficacy observed between murine studies and human trials (Geller et al., 2017). Moreover, the pancreatic tumor microbiome in patients has been implicated in modulating anti-tumor immunity. Higher intratumoral microbial diversity and the presence of certain bacteria correlate with enhanced immune cell infiltration and improved patient survival (Riquelme et al., 2019). For example, fecal microbiota transfer (FMT) from long-term PDAC survivors into KPC mice has been shown to increase tumor infiltration by cytotoxic T cells and slow tumor growth, underscoring the influence of microbial composition on anti-tumor immunity (Riquelme et al., 2019).

In addition to bacteria, fungal organisms have recently been recognized as oncological co-factors in PDAC. **Malassezia** species were found to colonize PDAC tumors and drive disease progression by activating the complement cascade (mannose-binding lectin pathway), an immune mechanism not accounted for in standard mouse models (Aykut et al., 2019). Clearance of these fungi or complement blockade mitigated tumor growth in preclinical studies, highlighting an immunosuppressive mycobiome element unique to the human condition (Aykut et al., 2019). The absence of this complex microbial ecosystem in murine models represents a critical gap, indicating that future translational approaches may need to incorporate microbiome-modulating strategies—such as targeted antimicrobials, FMT, or mycobiome–complement blockade—to mirror the human tumor milieu.

A second translational disparity lies in the genetic evolution and heterogeneity of tumors. Genetically engineered mouse models of PDAC (such as the KPC model) are driven by a predefined set of mutations and give rise to relatively genomically homogeneous tumors within a short timeframe (Hingorani et al., 2016). In contrast, human PDAC develops over many years, accumulating a more complex clonal architecture with extensive intra-tumoral heterogeneity (Makohon-Moore & Iacobuzio-Donahue, 2016). By the time of clinical presentation, human tumors may comprise multiple genetically distinct subpopulations, including clones that have acquired additional immune-evasive features through the process of cancer immunoediting (Balachandran et al., 2017). For instance, human PDAC tumors can undergo loss of MHC class I or **\beta2-microglobulin** as a means of escaping T cell surveillance in advanced disease (Balachandran et al., 2017). Such late-arising immune-resistant clones are seldom seen in short-term mouse tumors, which have fewer neoantigens and limited time for immune-driven selection. Elegant studies of long-term PDAC survivors have reinforced this: tumors from these rare patients contained neoantigens of unusually high quality, whereas most PDAC patients' tumors appeared to have selectively shed or silenced highly immunogenic neoantigens during their evolution (Balachandran et al., 2017).

Another challenge is the lack of robust predictive biomarkers in the clinic. Apart from the rare subset of MSI-high cases, pancreatic tumors generally do not possess known features that predict responsiveness to immune checkpoint blockade. Unlike in melanoma or lung cancer, neither high PD-L1 expression nor elevated tumor mutational burden stratifies PDAC patients for benefit from checkpoint inhibitors (Liang et al., 2018). Even histologic and transcriptomic markers of an immune-reactive tumor microenvironment (such as baseline CD8^+ T cell infiltration) have not yet been established as selection criteria in trials. Developing integrated biomarkers combining microbial taxa, antigen-presentation integrity, and TLS/CD8 programs remains an urgent priority to

identify which PDAC patients are likely to respond to immuno-oncology agents.

Finally, the translation of novel immunotherapeutic strategies has been impeded by unanticipated immune-related toxicities in patients. A prominent example is agonistic CD40 antibodies. In mouse PDAC models, CD40 agonists reversed immune suppression with manageable toxicity (Beatty et al., 2011). However, in human studies, these agents have shown a propensity to trigger cytokine release syndrome (CRS) and other systemic inflammatory reactions that were not evident in preclinical testing (Byrne et al., 2021). Similarly, the enzymatic stromal therapy PEGPH20 improved chemotherapy efficacy in KPC mice without significant adverse effects, yet clinical trials revealed that PDAC patients experienced a high rate of thromboembolic events (Hingorani et al., 2016). These examples underscore how certain immune-modulating therapies can introduce toxicity liabilities in humans—such as systemic cytokine storm or coagulopathy—that are not readily apparent in mouse models. Incorporating humanized mouse systems or in vitro human immune assays could help flag these potential toxicities early in development.

7. Future Directions and Unanswered Questions

Future research in PDAC must shift from broad stromal depletion to subtype-resolved reprogramming of the tumor microenvironment. While early clinical efforts to ablate the stroma failed or even accelerated disease, identifying the molecular programs that distinguish tumor-restraining from tumor-promoting CAF subsets remains a high priority. Key unanswered questions concern the stability of these CAF lineages and whether antigen-presenting CAFs (apCAFs) can be therapeutically induced to provide co-stimulation rather than inducing T-cell anergy (Section 2.1). Furthermore, disrupting the CXCL12–CXCR4 axis to prevent T-cell "corralling" in the stroma, combined with myeloid-repolarizing agents such as CD40 agonists or CSF1R inhibitors, represents a critical direction for converting "immune-excluded" phenotypes into "inflamed" ones (Section 4.2). Determining the optimal timing and sequencing of these interventions—specifically whether to prime the immune system before or during stromal remodeling—is essential to avoid compensatory myeloid influx and systemic toxicity.

A second major frontier involves overcoming the inherent low immunogenicity and antigen-presentation deficits that characterize most PDAC tumors. Beyond the rare MSI-H subset, future studies should investigate whether non-canonical antigens, such as spliced peptides or epigenetic neoantigens, can serve as viable targets for TCR-engineered T cells or personalized mRNA vaccines (Section 5.3). Mechanistically, it is vital to determine how autophagy-mediated MHC-I degradation is initiated and whether lysosomal inhibitors can reliably restore surface antigen expression in vivo (Section 2.3). Strategies to expand Batf3-dependent conventional dendritic cell (cDC1) populations via Flt3L or STING agonists are also essential to ensure robust cross-priming. Breakthroughs in this area will likely require "triplet" regimens that simultaneously enhance antigen supply, restore presentation machinery, and relieve the checkpoint-mediated exhaustion of newly recruited T cells.

Addressing the metabolic and microbial drivers of immune evasion constitutes an emerging area of high priority. Future research should utilize spatially resolved metabolomics to map the distribution of adenosine, kynurenine, and lactate across hypoxic niches to determine which TME compartments are the dominant sources of metabolic suppression (Section 2.4). Additionally, the role of the intratumoral microbiome in modulating both chemotherapy efficacy and local immunity requires deeper mechanistic mapping to determine if targeted antimicrobials or fecal microbiota transplants can improve therapeutic responses (Section 6.1).

Finally, closing the gap between preclinical success and clinical failure requires next-generation models with higher translational fidelity. While KPC mice have been foundational, they often fail to

capture the genetic heterogeneity and late-stage immune editing seen in humans. The development of autologous humanized-PDX "avatars" and the identification of robust biomarkers—such as the presence of tertiary lymphoid structures (TLS) or specific myeloid signatures—will be necessary to move toward patient-stratified immunotherapy. Ultimately, the goal is to transition from universal treatment protocols to rational, multi-modal combinations that address the unique, redundant layers of immunosuppression present in each patient's tumor.

8. Conclusion

The investigation into the immune landscape of PDAC reveals a malignancy defined by its extraordinary capacity for immune evasion. As synthesized in this review, the resistance of PDAC to conventional immunotherapy is not the result of a single defect but rather the product of a multilayered, redundant system of suppression. This "immune-privileged" state is enforced by a dense desmoplastic stroma that acts as both a physical and biochemical barrier, a dominant population of immunosuppressive myeloid cells and regulatory T cells, and a metabolic environment characterized by hypoxia and nutrient depletion. Furthermore, the tumor-intrinsic lack of immunogenicity—driven by low mutational burden and the active downregulation of antigen-presentation machinery—ensures that even when the physical barriers are breached, the adaptive immune system often fails to recognize the neoplastic cells as targets.

The integration of findings from diverse preclinical platforms has been instrumental in mapping these complex interactions. Genetically engineered mouse models, such as the KPC strain, have provided high-fidelity insights into the spontaneous evolution of the PDAC microenvironment, while syngeneic and humanized systems have allowed for the rapid testing of novel therapeutic interventions. However, the transition from preclinical success to clinical efficacy remains the most significant hurdle in the field. While mouse models have successfully identified targets like CSF1R, CD40, and various metabolic checkpoints, the clinical translation of these findings has often been met with modest response rates or unanticipated toxicities. This disconnect underscores the profound influence of factors often absent in standard murine models, including the intratumoral microbiome, the extensive genetic heterogeneity of advanced human disease, and the compensatory mechanisms that allow the tumor to adapt when a single suppressive pathway is inhibited.

The broader significance of this research area lies in the shift away from monotherapeutic approaches toward rational, multi-modal combination strategies. The clinical data reviewed here demonstrate that while immune checkpoint inhibitors are largely ineffective as single agents in microsatellite-stable PDAC, they may yet find a role as components of "triplet" or "quadruplet" regimens. By simultaneously priming T-cell responses with vaccines or adoptive therapies, reprogramming the myeloid compartment with agonists, and dismantling metabolic or stromal barriers, it may be possible to convert the "cold" PDAC microenvironment into one that is permissive to anti-tumor immunity. The rare but durable responses seen in MSI-high patients and the emerging success of personalized neoantigen vaccines provide a proof-of-concept that the PDAC immune barrier is not entirely impenetrable.

In summary, while PDAC remains one of the most difficult cancers to treat with immunotherapy, the collective body of research from murine models and human trials has provided a clear roadmap of the obstacles that must be overcome. The complexity of the PDAC tumor microenvironment necessitates a sophisticated, subtype-aware approach to therapy that accounts for the unique biological context of each patient. Ultimately, bridging the gap between the bench and the bedside will require a continued commitment to high-fidelity modeling and the iterative refinement of clinical trial designs to address the resilient nature of this lethal disease.

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