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Review

CD40 Agonism in Pancreatic Ductal Adenocarcinoma: Expression, Biology, and Therapeutic Targeting

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains highly lethal and is largely refractory to immune checkpoint inhibition, reflecting limited antigen-specific priming, dominant myeloid suppression, dense desmoplasia, and abnormal vasculature that together enforce immune exclusion. CD40 is a central costimulatory receptor that links $CD4^+$ T cell help (via CD40L/CD154) to antigen-presenting cell (APC) licensing and effective $CD8^+$ T cell priming, positioning CD40 agonism as a rational strategy to enhance antitumor immunity in PDAC. In addition to robust expression on APC populations (DCs, macrophages, and B cells), CD40 has been reported on subsets of PDAC tumor cells and stromal compartments (including fibroblasts and endothelial cells), implying that CD40-directed therapies may engage multiple cellular nodes and influence both immune activation and microenvironmental remodeling. Mechanistically, CD40 signaling integrates TRAF-dependent pathways (canonical/non-canonical NF-κB, MAPK, and PI3K/AKT) that can promote APC maturation, IL-12-associated Th1 programming, macrophage repolarization, and matrix remodeling that may reduce physical barriers to immune infiltration; however, tumor-intrinsic CD40 signaling can be context-dependent and has been linked to divergent survival or apoptotic outcomes in different settings. Clinically, multiple CD40 agonists are in development, predominantly in combination regimens with chemotherapy, checkpoint blockade, and/or vaccine platforms, with evidence of pharmacodynamic immune engagement but variable efficacy and incomplete randomized validation. Baseline CD40 expression has not consistently predicted benefit, underscoring the need for spatially resolved profiling and on-treatment pharmacodynamic biomarkers to guide patient selection, sequencing, and regimen optimization. Ongoing studies and next-generation, tumor-localized agonist formats will define whether CD40 agonism can deliver consistent, durable clinical benefit in PDAC.

Keywords: pancreatic cancer (PDAC) CD40-agonists; tumor microenvironment; immunotherapy

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the predominant subtype of pancreatic cancer and is the sixth leading cause of cancer mortality worldwide [1]. Its incidence is rising, and projections suggest that it will become the second leading cause of cancer-related death by 2030 [2]. Standard

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combination chemotherapy regimens (e.g., FOLFIRINOX or gemcitabine/nab-paclitaxel) provide limited long-term benefit [3,4], with 5-year survival remaining around 13% [5], reinforcing the need for new therapeutic strategies.

Immune checkpoint inhibitors (ICIs) alone have transformed care in several solid tumors [6–14], but have shown limited activity in biomarker-unselected PDAC [15–17]. This refractoriness is largely attributed to an immunosuppressive tumor microenvironment (TME) enriched for tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells, combined with a dense desmoplastic stroma and abnormal, poorly perfused vasculature that restricts immune trafficking and drug delivery. Together with low tumor mutational burden and sparse tumor-specific effector T cells and DCs, these features contribute to an immunologically "cold" phenotype [18–22]. Therapeutic strategies in PDAC, therefore, likely require both improved antigen presentation and T cell priming, as well as relief of stromal-myeloid constraints on effector function.

CD40, a member of the tumor necrosis factor receptor (TNFR) superfamily, is a central co-stimulatory receptor in adaptive immunity that links $CD4^+$ T cell help to antigen-presenting cell (APC) licensing and downstream $CD8^+$ T cell priming [23,24]. Its physiological ligand, CD40L (CD154), is transiently upregulated on activated $CD4^+$ T cells and can also be provided in soluble form, including from platelets, enabling CD40 signaling in both immune and vascular compartments [25,26]. CD40 is primarily expressed by APCs, including dendritic cells (DCs), macrophages, and B cells [25–27]. Engagement by CD40L delivers a potent activation signal that promotes APC maturation, cytokine production, and enhanced cross-presentation, thereby strengthening T cell priming and downstream effector functions [23–28]. CD40 expression has also been reported on subsets of PDAC tumor cells, with heterogeneity across tumors and disease stages, suggesting context-dependent roles in tumor biology and immune interactions [29,30]. Additionally, CD40 has been detected in stromal compartments, including cancer-associated fibroblasts (CAFs) and select endothelial populations, supporting the concept that CD40-directed therapies may exert effects beyond classical APC activation [24,30–33].

Consistent with this biology, CD40 agonism in preclinical PDAC models can induce immune activation and stromal remodeling [31,32]. Several CD40 agonists are currently in clinical trials, showing signs of immune activation and generally acceptable safety profiles across various solid tumors, including PDAC. [34–36]. However, CD40 signaling is context-dependent: while APC-directed activation is typically immunostimulatory, tumor-intrinsic CD40 signaling has been associated with pro-survival or pro-growth programs in some settings, with potential implications for therapeutic design and patient selection [37–39].

In this review, we summarize CD40 expression patterns and measurement considerations, explore CD40 signaling and cell-specific roles in PDAC, and evaluate the clinical and translational progress of CD40-directed strategies. We emphasize how combination regimens and appropriate sequencing may be required to overcome PDAC immune resistance and enable more consistent therapeutic responses.

2. CD40 Expression Landscape in PDAC

In PDAC, CD40 expression is most prominently detected on immune cells but is also reported on tumor cells and stromal elements, with variable levels across compartments and disease settings. This pattern aligns with CD40's roles in immune activation, stromal remodeling, and tumor biology.

2.1. CD40 Expression on Non-Immune PDAC Compartments

Beyond immune cells, CD40 is present across multiple non-immune compartments in PDAC, including tumor cells and stromal elements. A substantial subset of PDAC tumor cells expresses CD40 (reported at around 68% across cohorts) [29,30], but expression is markedly heterogeneous within tumors and is often higher in advanced or metastatic disease [29]. Functionally, tumor-intrinsic CD40 signaling can yield divergent outcomes, ranging from apoptosis to enhanced proliferation and survival, depending on cellular context, with NF-κB and MAPK pathways

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implicated as key downstream effectors [37–39]. Tumor-cell CD40 expression has also been reported to impair T cell activation by reducing T cell CD154 (CD40L) expression and suppressing cytokine production and proliferation, consistent with a potential contribution to immune escape [40]. This heterogeneity likely reflects differences in oncogenic programs, local cytokine milieus, and spatiotemporal variation between primary tumors and metastatic sites, where immune and stromal architecture can differ and may influence responsiveness to CD40 agonism [41,42]. In the stroma, CD40 expression on subsets of cancer-associated fibroblasts (CAFs) and endothelial cells links CD40 signaling to extracellular matrix (ECM) remodeling and vascular regulation. CAF CD40 activation can trigger cytokine and growth factor production that influence matrix metalloproteinase (MMP) activity, leading to ECM remodeling and effects on invasion that depend on CAF subtype and context [33,43–45]. Additionally, CD40-expressing endothelial cells participate in inflammatory vascular remodeling and angiogenic processes [25,26,39,46,47]. Crosstalk between CAFs and endothelial cells, mediated by factors like vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), can result in hypovascularity, reduced drug delivery, and immune exclusion [48–51]. Consistent with these compartmental effects, studies show that agonistic CD40 activation can induce stromalysis via proteases produced by activated myeloid cells, degrading dense ECM and facilitating infiltration of DCs and effector T cells into tumor tissue [31,32,43]. Collectively, these observations support the concept that CD40-directed therapies may act not only through APC activation but also via tumor-intrinsic and stromal/vascular programs that shape PDAC immune resistance and treatment sensitivity.

2.2. CD40 Expression on Immune Cells Within the PDAC Tumor Microenvironment

The most robust expression of CD40 in PDAC is observed on APC populations, including DCs, macrophages (especially TAMs), monocytes, and B cells [25–27]. DC subsets implicated in cross-priming, including cDC1 and cDC2 populations (including $CD141^+$ DCs) and $LAMP3^+$ mature/regulatory DC states, depend on CD40 engagement for functional maturation and for efficient priming of cytotoxic $CD8^+$ T cells [23,26,52]. However, their function in PDAC is frequently inhibited by tumor-derived cytokines and suppressive signals from myeloid populations [53,54].

TAMs, often skewed toward an M2-like phenotype, express variable levels of CD40 [55]. Engagement of CD40 has been shown to promote M1-like inflammatory reprogramming with increased antigen presentation, pro-inflammatory cytokine production (e.g., IL-12, TNF-α), and induction of matrix-degrading programs that can facilitate immune cell infiltration [43]. Within tertiary lymphoid structures (TLS), $CD40^+$ B cells may contribute to local antigen presentation and support T cell priming, and, in mature TLS, can undergo antigen-driven maturation and plasma cell differentiation with local antibody production[56]. Importantly, T cells are key providers of the CD40 signal: activated $CD4^+$ T cells upregulate CD40 ligand (CD40L/CD154), which licenses $CD40^+$ APCs and supports broader lymphocyte activation [24,25,28]. CD40 expression has also been described on subsets of MDSCs, where CD40 engagement may modulate suppressive or effector functions in a context-dependent manner [57–59]. NK cells, on the other hand, express CD40 ligand and not the CD40 receptor itself. CD40L engagement on NK cells activates them, enabling them to recognize and kill CD40-expressing target T cells, including tumor cells, and is regulated by IL-12 and IFNγ [60,61]. This activates the NF-κB signaling, resulting in cytotoxic activity [62,63].

3. Biological Functions and Signaling Pathways of CD40 in PDAC

The biological consequences of CD40 engagement are compartment-dependent, shaped by the cellular source of CD40 and the local availability and form of its ligand. The key cellular and molecular mechanisms engaged by CD40 agonists in PDAC are summarized in **Figure 1.**

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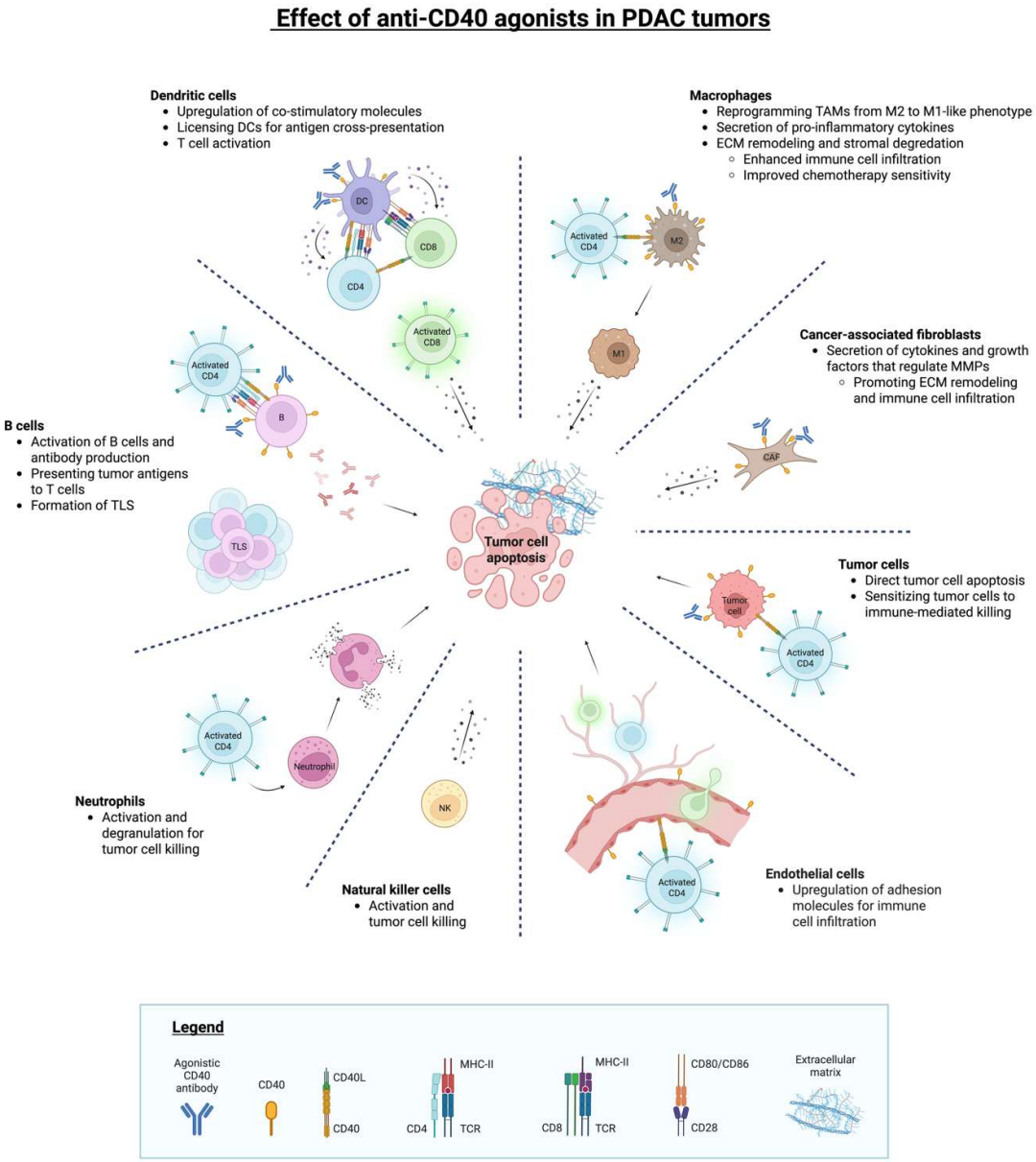

Figure 1. Expression of CD40 and effect of agonistic CD40 antibodies in pancreatic cancer.

3.1. Core CD40 Signaling Pathways Shaping Immune Activation

CD40 is a TNFR family receptor that lacks intrinsic enzymatic activity and signals by recruiting TNF receptor-associated factor (TRAF) adaptor proteins after ligand-induced receptor clustering. The composition and stoichiometry of TRAFs bias downstream pathway engagement and functional output [64–66]. A principal consequence of CD40 ligation is activation of canonical NF-κB signaling, inducing transcriptional programs that support APC maturation and T cell priming, including cytokines (e.g., IL-6, TNF-α), adhesion molecules (e.g., ICAM-1), and costimulatory regulators within the B7 family [66,67].

Among the latter, CD40 engagement upregulates the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) on DCs and B cells, and enhances T cell activation [23,28,68,69]. Interestingly, CD40 activation has also been shown to upregulate B7-H3 (CD276) expression on DCs, and this upregulation was functionally required for an enhanced antitumor immune response induced by CD40-activated, tumor antigen-pulsed DCs [66,70,71]. The B7-H3 checkpoint molecule is a member

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of the B7 family and is overexpressed in various solid malignancies, including PDAC, where it promotes tumor growth, metastasis, and treatment resistance [72–74]. Therefore, it has emerged as a potential target in cancer immunotherapy. Recent advances in B7-H3-targeting antibody-drug conjugates (ADCs), such as YL201 and HS-20093 (GSK5764227), provide a promising approach for antitumor therapy in early-phase clinical trials [75,76]. Whether CD40 agonist-mediated upregulation of B7-H3 on myeloid cells within the TME could enhance the efficacy of B7-H3-directed ADCs, or, conversely, serve as an immune evasion mechanism that warrants therapeutic targeting, remains an open question with important implications for rational combination strategies that have not yet been addressed.

CD40 can also engage the non-canonical NF-κB axis, contributing to more sustained programs relevant to APC function and lymphoid organization [77]. In parallel, CD40 activates mitogen-activated protein kinase (MAPK) cascades, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, which shape cytokine production, differentiation, and survival programs across myeloid and stromal cells [66,70,71]. CD40 engagement additionally activates the phosphoinositide 3-kinase (PI3K)/AKT pathway, supporting cellular metabolism and antigen-processing capacity in APCs and modulating survival signaling in a context-dependent manner [78]. JAK3-STAT5 signaling is generally not a primary, direct CD40 pathway but can be engaged indirectly via autocrine/paracrine cytokines induced downstream of CD40 activation, thereby tuning inflammatory polarization and preventing tolerogenic programming in APCs [79–82]. Collectively, these signaling modules determine the magnitude, durability, and qualitative balance of CD40-driven immunity, and they provide a mechanistic basis for why CD40 agonism can have distinct effects across PDAC cellular compartments.

Targeting the JAK/STAT pathway has emerged as a potential therapeutic strategy in cancer, as it is involved in inflammatory responses and progression, particularly STAT3 in pancreatic cancer [83–90]. In a randomized, double-blind, phase II trial in patients with metastatic pancreatic cancer, a subgroup with increased systemic inflammation demonstrated signs of clinical benefit after treatment with ruxolitinib, a JAK1/JAK2 inhibitor, plus capecitabine chemotherapy [91]. Encouraged by these findings, two phase III randomized, placebo-controlled trials, JANUS 1 (NCT02117479) and JANUS 2 (NCT02119663), were initiated to evaluate the efficacy of this combination therapy [92]. Patients with advanced or metastatic pancreatic cancer and increased systemic inflammation, with one prior chemotherapy regimen, received ruxolitinib plus capecitabine or a placebo plus capecitabine. Unfortunately, both trials were terminated prematurely due to futility after interim analysis of JANUS 1, as the combination therapy failed to improve survival compared to placebo. Further investigation into JAK/STAT inhibition was pursued in another phase Ib/II study, in which itacitibin, a selective JAK1 inhibitor, was combined with gemcitabine and nab-paclitaxel in patients with treatment-naïve advanced metastatic solid tumors, including PDAC [93]. Despite demonstrating some clinical activity with an overall response rate of 24%, this trial was also terminated following the disappointing results from JANUS 1 and 2.

Given that CD40 agonism can indirectly engage JAK3-STAT5 signaling [79–82], combining CD40 agonists with JAK/STAT pathway inhibitors could offer a novel therapeutic approach to further enhance the antitumor response in PDAC. While CD40 engagement promotes APC maturation and enhanced cross-presentation for T cell priming and activation, simultaneously inhibiting the JAK/STAT pathway in the tumor might reduce progression and increase chemotherapy sensitivity, thereby enhancing antitumor efficacy and increasing survival. However, JAK/STAT pathway inhibitors may also have immunosuppressive effects [94–96]. Therefore, a comprehensive understanding of how JAK/STAT signaling interacts with CD40-driven immune activation and the overall effect on the immune system and TME could provide valuable insights into this combination strategy. Whether JAK/STAT pathway inhibitors would enhance or impair the efficacy of CD40 agonists, and whether the toxicity profile would be acceptable when combining these two regimens, remains an open question that warrants further preclinical investigation.

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3.2. The CD40-CD40L Axis In Vivo: Ligand Sources and Signaling Context

Physiologic CD40 activation is mediated by CD40L (CD154), which is transiently upregulated on activated $CD4^+$ T cells and provides the canonical help signal required for APC licensing and effective cytotoxic T cell priming [23,25,26,28]. CD40L can also be supplied by other activated immune cells and is present in soluble form, most prominently derived from platelets, supporting CD40 signaling in vascular and inflammatory contexts and contributing to endothelial activation programs [25,26,39,46,47]. The biologic outcome of CD40 engagement is therefore shaped not only by CD40 expression but also by where, when, and in what form CD40L is available (cell-bound vs soluble), as well as by the inflammatory milieu that determines pathway integration (e.g., NF-κB/MAPK tone) [71,97]. In PDAC, where productive $CD4^+$ T cell help is often limited and myeloid suppression is prominent, therapeutic CD40 agonism aims to bypass inadequate endogenous CD40L delivery and restore licensing signals needed for effective priming and intratumoral immune activation [23,26,69].

3.3. CD40-Driven Activation of Antigen-Presenting Cells

CD40 engagement on DCs, macrophages, and B cells induces maturation and functional activation, thereby bridging innate and adaptive immunity. Upon CD40 ligation via CD40L or agonistic antibodies, DCs upregulate MHC class I/II and enhance cross-presentation, thereby enabling priming of both $CD4^+$ and $CD8^+$ T cells [23,26]. CD40 activation also increases CD80/CD86 expression on DCs, ensuring delivery of the necessary secondary signals to naive T cells, promoting their proliferation and differentiation into cytotoxic and helper subsets [23,69]. In addition, CD40 activation promotes IL-12 production, supporting Th1 polarization, $CD8^+$ T cell expansion, and IFN-γ-associated effector programming, all of which are critical for antitumor responses [23,26,43,98]. These changes promote robust cross-priming of $CD8^+$ T cells, a process profoundly deficient in untreated PDAC [21,99–101]. Therapeutic CD40 agonists could restore this critical antitumor axis.

3.4. Reprogramming of Tumor-Associated Macrophages

The PDAC TME favors immunosuppressive TAM states driven by chronic inflammation, hypoxia, and stromal cues [55,102–104]. CD40 signaling can reprogram M2-like macrophages toward a more inflammatory, antigen-presenting phenotype, associated with increased IL-12/TNF-α production, enhanced antigen presentation, induction of matrix-degrading programs, and improved immune cell recruitment [31,32,43,105]. In preclinical PDAC models, macrophage reprogramming is a major contributor to CD40 agonist activity and is mechanistically linked to improved T cell infiltration and intratumoral activation [31,106,107].

3.5. Stromal and Vascular Remodeling Downstream of CD40 Activation

CD40 agonism can modify PDAC stromal barriers through myeloid-driven remodeling programs [32]. CD40 activation increases expression of multiple matrix metalloproteinases (MMPs) in inflammatory macrophages and can suppress profibrotic CAF activity through paracrine and contact-dependent signaling, reducing ECM deposition and facilitating immune trafficking and drug penetration [31,32,43]. Endothelial cells express CD40, whose expression is notably upregulated by pro-inflammatory stimuli such as TNF-α and IFN-γ [46,108]. The interaction between endothelial CD40 and its ligand (CD40L), derived from activated T cells, monocytes, or platelets, triggers a potent pro-inflammatory response in the endothelium [46,47,109,110]. This activation leads to the expression of adhesion molecules (e.g., E-selectin, ICAM-1, VCAM-1), the production of inflammatory cytokines (IL-1, IL-6, IL-8), chemokines, MMPs, and increased procoagulant activity [46,109,111–113]. In PDAC models, inflammatory remodeling and stromal loosening have been linked to increased DC and T cell infiltration, supporting a mechanistic role for CD40 in overcoming physical and cellular barriers to immunity [24,31,43,105,114]. Furthermore, CD40-CD40L interactions can induce the production of angiogenic factors, including VEGF and fibroblast growth factor, with soluble CD40L promoting

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endothelial cell growth [115–117]. However, the function of CD40 in tumor angiogenesis is complex. Some studies indicate that CD40 activation may enhance tumor neoangiogenesis by increasing VEGF levels [118,119], whereas others show reduced tumor blood vessels and slower tumor growth due to inflammatory remodeling [39,120].

3.5. Tumor-Intrinsic CD40 Signaling in PDAC

In subsets of PDAC, CD40 is expressed by malignant epithelial cells and can transmit tumor-intrinsic signals upon ligation [29,30,121]. Downstream activation of NF-κB and MAPK pathways has been linked to divergent phenotypes, ranging from apoptosis [29,122,123] to enhanced proliferation and survival [121,124–126], depending on cellular state and microenvironmental context [125,127,128]. Beyond effects on tumor cell fitness, tumor-cell CD40 signaling has also been reported to modulate antitumor immunity, including attenuating T cell activation through reduced T cell CD154 (CD40L) expression and impairing cytokine production and proliferation in some settings [40,121,129]. These context-dependent tumor-intrinsic programs are relevant to therapeutic CD40 agonism because the net biologic effect may reflect the balance between APC licensing and myeloid reprogramming on one hand and tumor-cell-associated signaling on the other hand [27,33,130]. Accordingly, interpretation of CD40-targeted strategies in PDAC should consider not only immune compartment engagement but also tumor-cell CD40 expression and downstream signaling competence.

4. Therapeutic Targeting of CD40 in PDAC

CD40 agonism is being pursued in PDAC to compensate for insufficient endogenous CD40L-driven APC licensing and to induce coordinated programs that are typically deficient in this disease: improved antigen presentation and T cell priming, myeloid reprogramming, and remodeling of stromal/vascular barriers that limit immune trafficking and drug delivery. Because CD40 pathway engagement can be pharmacodynamically robust yet clinically variable as monotherapy, most development has focused on rational combinations and on defining the settings and biomarkers that best capture effective pathway engagement.

4.1. CD40 Agonistic Modalities and Design Principles

Productive CD40 signaling requires receptor clustering; therefore, in vivo agonism depends on the efficiency with which an agent promotes CD40 multimerization. This is influenced by the targeted CD40 epitope, effective valency, and, particularly for agonistic monoclonal antibodies (mAbs), Fcγ receptor (FcγR)-mediated crosslinking on accessory cells [131]. These parameters (isotype/Fc engineering, FcγR engagement profile, and binding geometry) shape the magnitude and tissue distribution of immune activation and contribute to inter-agent differences in tolerability and optimal dosing schedules. Accordingly, CD40 agonists should not be treated as a uniform class: selicrelumab (RG7876/CP-870,893), SEA-CD40, mitazalimab (JNJ-64457107/ADC-1013), sotigalimab (APX005M), CDX-1140, ChiLob 7/4, and LVGN7409 are all under clinical investigation in pancreatic cancer (Table 1). While each aims to mimic CD40L-driven activation, they differ in molecular properties that can alter clustering requirements and downstream biology. Across agents, monotherapy activity in PDAC has generally been modest, reinforcing the rationale for combination strategies that provide antigen release and/or relieve downstream suppression.

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Table 1. Monoclonal CD40 agonists utilized in clinical trials with pancreatic cancer.

Agent	Other names	Developer	Antibody class	Fc engineering
Sotigalimab	APX005M	Apexigen / Pyxis Oncology	IgG1	Enhanced FcγRIIB binding
Mitazalimab	JNJ-64457107/ADC-1013	Alligator Bioscience	IgG1	FcγR crosslinking-dependent; no specific Fc engineering reported
Selicrelumab	RG7876/CP-870,893	Pfizer / Genentech / Roche	IgG2	None
CDX-1140	-	Celldex Therapeutics	IgG2	None
ChiLob 7/4	-	University of Southampton	IgG1	None
SEA-CD40	-	Seagen	IgG1	Non-fucosylated Fc; Enhanced FcγRIIIa binding
LVGN7409	-	Lyvgen Biopharma	IgG1	Selective FcγRIIB binding

4.2. Combination Approaches with Chemotherapy

Chemotherapy establishes a mechanistic foundation for CD40 activation in PDAC by boosting antigen availability through tumor cell death and antigen release, promoting immunogenic cell death signals, and altering myeloid cell composition. Additionally, CD40-induced remodeling of myeloid and stromal components may enhance stromal and vascular permeability, facilitating better access for immune cells and drugs to the tumor tissue [32,106,132]. A key goal of chemo-CD40 therapy is to reprogram TAMs, including $CD163^+$ myeloid cells, to adopt inflammatory, antigen-presenting, and matrix-remodeling phenotypes, shifting the tumor microenvironment from immunosuppressive to one more supportive of effector functions. This is important because high $CD163^+$ macrophage levels correlate with poorer outcomes across various cancers [99,133–135]. Preclinical data support a synergistic effect between chemotherapy and CD40 activation, showing improved T cell priming and longer survival in experimental models [32,106,132].

Clinically, combinations of chemotherapy and CD40 in PDAC have been examined, including gemcitabine, gemcitabine combined with nab-paclitaxel, and mFOLFIRINOX (Table 2). In a phase I trial, patients with chemotherapy-naïve advanced PDAC received selicrelumab (CP-870893) in combination with gemcitabine, resulting in an ORR of 19% (4 of 22 patients). The median PFS was 5.2 months (95% CI: 1.9-7.4), and the median OS was 8.4 months (95% CI: 5.3-11.8), with a 1-year OS rate of 28.6%. Immune activation was observed through increased inflammatory cytokines and B cell expression of costimulatory molecules [136].

Table 2. Completed clinical trials using monoclonal CD40 agonists in pancreatic cancer.

Regimen	Design	Tumor type	Treatment scheme	Clinical outcomes	Immunological outcomes	Toxicity	Clinical trial/ reference
ChiLob 7/4	Phase I	CD-40 expressing solid tumors and diffuse large B cell lymphoma (DLBCL), including 2 pancreatic cancer patients (total n=28)	ChiLob7/4 weekly for 4 doses	No objective responses. Disease stabilization in 15/29 treatments (52%), with median duration of 6 months.	Signs for immune activation and effector cell trafficking.	Well-tolerated. One DLT. Infusion reactions could be prevented with single-dose corticosteroid premedication.	NCT01561911 [98]
SEA-CD40	Phase I	Advanced solid tumors (n=56) and lymphoma (n=11),	SEA-CD40 monotherapy in 21-day cycle.	One CR and three SD in seven	Cytokine induction and activation of T	Acceptable safety profile. Infusion/hypersen	NCT02376699 [193]

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Regimen	Design	Tumor type	Treatment scheme	Clinical outcomes	Immunological outcomes	Toxicity	Clinical trial/ reference
		including 3 pancreatic cancer patients (total n=67)		lymphoma patients.	cells and NK cells in the peripheral blood	sitivity reactions (IHRs) in 73% of the patients, primarily grade 1-2.	
CP-870,893 (=selicrelumab) + gemcitabine	Open-label, dose-escalation, phase I	Chemotherapy-naive patients with advanced PDAC (n=22).	Gemcitabine once weekly for three weeks with CP-870893 48 hours after gemcitabine on day three of each 28-day cycle.	Median PFS 5.2 months (95% CI: 1.9-7.4). Median OS 8.4 months (95% CI: 5.3-11.8). 1-year OS 28.6%. ORR 19% (4/22 patients).	Increase in inflammatory cytokines. Increase in B cell expression of costimulatory molecules. Transient depletion of B cells.	Well-tolerated. One DLT. Most common AE was CRS (grade 1 to 2).	NCT00711191 [140]
Selicrelumab +/- Gem/Nab	Open-label, phase I	Resectable PDAC (n=16).	Arm I (n=16): neoadjuvant selicrelumab two weeks prior to surgery. Arm II (n=11): neoadjuvant Gem/Nab followed by selicrelumab two days later, prior to surgery. Adjuvant Gem/Nab followed by selicrelumab two days later, up to four 28-day cycles, in both arms.	Both arms (n=16): Median OS 23.4 months (95% CI: 18.0-28.8). Median DFS 13.9 months (95% CI: 2.9-24.8). Arm I: Median OS 23.4 months (95% CI: 9.1-37.6). Median DFS 9.8 months (95% CI: 0.4-19.2). 1-year DFS 49.9%. 1-year OS 81.8%. Arm II: Median OS and DFS are not reached. 1-year DFS 75.0%. 1-year OS 100%.	82% of the treated tumors were T cell enriched. More active and proliferative T cells in both TME and circulation. Reduced tumor fibrosis. Less M2-like macrophages. More mature intratumoral DCs. Systemically increased inflammatory cytokines.	Acceptable toxicity profile. Selicrelumab related AEs were mostly mild; 5 patients with grade 3 AEs, and one patient with grade 4 AE. Three SAEs observed in two patients.	NCT02588443 [43]
Mitazalimab + mFOLFIRINOX (OPTIMIZE-1)	Single-arm, phase Ib/II	Chemotherapy-naive patients with metastasized PDAC (n=70)	During the first 21 day treatment cycle, mitazalimab on day 1 (priming dose) and on day 10, and mFOLFIRINOX on day 8. During subsequent 14-day treatment cycles, mFOLFIRINOX on day 1 and mitazalimab on day 3.	Median PFS 7.7 months (95% CI: 5.8-11.3). Median OS 14.3 months (95% CI: 10.0-21.6). 1-year PFS 34%. 1-year OS 59%. ORR 40% (23/57 patients).	Mitazalimab-induced increases in activated circulating myeloid, B cell, and T cell frequencies correlate with better outcomes. Intratumoral myeloid and T cell activation in objective responders.	Manageable safety. One DLT observed. Most common grade 3 or worse AEs: neutropenia 26%, hypokalaemia 16%, anaemia and thrombocytopenia 11%. SAEs in 41%, none considered related to mitazalimab. No treatment-related deaths.	NCT04888312 [136,139]

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Regimen	Design	Tumor type	Treatment scheme	Clinical outcomes	Immunological outcomes	Toxicity	Clinical trial/ reference
Sotigalimab (APX005M) + Gem/nab +/- nivolumab (PRINCE)	Non-randomized, open-label, four cohort, phase Ib	First line treatment for metastasized PDAC (n=30)	1) Nivolumab + Gem/Nab, 2) Sotigalimab + Gem/Nab, 3) Sotigalimab + nivolumab + Gem/Nab. Nivolumab on days 1 and 15. Sotigalimab on day 3 (=2 days after chemotherapy), or on day 10 if patients received chemotherapy on day 8.	Median PFS 11.7 months (95% CI: 7.1-17.8). Median OS 20.1 months (95% CI: 10.5-not estimable). ORR 58% (14/24 DLT-evaluable patients).	Decrease of naïve B cells and increase of plasmablasts. Increased frequency of CD141-negative myeloid DCs and pDCs. Increased proportions of activated $CD8^+$ and $CD4^+$ T cells. Increased proportions of CD4+ naïve, central memory, and regulatory T cells. Decreased *KRAS* VAF (in 12/14 patients who had detectable KRAS mutations in plasma).	Treatment is tolerable. Two DLTs (grade 3 and 4 febrile neutropenia), however deemed unrelated to either sotigalimab or nivolumab. 14 (47%) patients with treatment-related SAE. Most common were pyrexia, sepsis, haemolytic uraemic syndrome, and nausea. Overall, grade 3 or 4 treatment-related adverse events occurred in 28 (93%) of 30 patients and were clinically manageable. Most common grade 3-4 treatment-related AEs were haematological and generally transient (lymphocyte count decrease, neutrophil count decrease, and anaemia). No grade 3-4 CRS and infusion reactions. Two deaths due to AEs related to Gem/Nab (sepsis and septic shock in the setting of neutropenia). One death from an unknown cause occurring 4 months after the last study intervention.	NCT03214250 [137]

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Regimen	Design	Tumor type	Treatment scheme	Clinical outcomes	Immunological outcomes	Toxicity	Clinical trial/ reference
Sotigalimab (APX005M) + Gem/Nab +/- nivolumab (PRINCE)	Randomized, open-label, phase II	First line treatment for metastasized PDAC (n=105)	1) Nivolumab + Gem/Nab, 2) Sotigalimab + Gem/Nab, 3) Sotigalimab + nivolumab + Gem/Nab. Nivolumab on days 1 and 15. Sotigalimab on day 3 (=2 days after chemotherapy), or on day 10 if patients received chemotherapy on day 8.	1) Nivo/chemo (n=34): Median PFS 6.4 months (95% CI: 5.2-8.8). Median OS 16.7 months (95% CI: 9.8-18.4). 1-year OS 57.7% ORR 50% (95% CI: 32-68). 2) Sotiga/chemo (n=36): Median PFS 7.3 months (95% CI: 5.4-9.2). Median OS 11.4 months (95% CI: 7.2-20.1) 1-year OS 48.1% ORR 33% 3) Sotiga/nivo/chemo (n=35): Median PFS 6.7 months (95% CI: 4.2-9.8). Median OS 10.1 months (95% CI: 7.9-13.2). 1-year OS 41.3% ORR 31%.		98% of the patients had at least one treatment related AE. Most common grade 3-4 treatment related AEs were hematologic and generally transient. Two patients died due to an AE. One from acute hepatic failure possibly related to sotiga/chemo. One from intracranial haemorrhage possibly related sotiga/nivo/chemo.	NCT03214250 [138]
Mitazalimab + autologous DC vaccine (REACtiVe-2)	Open-label, dose-escalation, phase I	Metastasized PDAC (n=16)	Biweekly 25 × 10[6] DCs (1/3 i.d. and 2/3 i.v.) co-administered with mitazalimab for the first three administrations, followed by a fourth and fifth administration if no disease progression.	In patients without PD at baseline: Median PFS 2.76 months (IQR: 2.40-6.86), and median OS 12.1 months (IQR: 5.74-21.77). 1-year PFS rate 13% 1-year OS rate 50%. No objective radiological response. 8/16 patients (50%) with SD after 3x administrations.	Systemic increase in activated and vaccine-specific T cell responses. Increased T cell infiltration and decreased collagen deposition in post-treatment biopsies.	Safe and well-tolerated. One transient DLT (grade 3 fever).	NCT05650918 [155]

In a separate phase I study, sixteen patients with resectable PDAC received neoadjuvant selicrelumab, with or without gemcitabine/nab-paclitaxel, followed by adjuvant gemcitabine/nab-paclitaxel and selicrelumab. The median DFS and OS for the neoadjuvant selicrelumab group (n=11) were 9.8 months (95% CI: 0.4-19.2) and 23.4 months (95% CI: 9.1-37.6), respectively. For the group receiving both selicrelumab and gemcitabine/nab-paclitaxel (n=5), median DFS and OS were not

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reached. When combining both groups, median DFS was 13.9 months (95% CI: 2.9-24.8), and median OS was 23.4 months (95% CI: 18.0-28.8). Patients treated with selicrelumab showed a T cell-enriched tumor microenvironment (82% vs. 37% in untreated tumors, P=0.004), featuring more active and proliferative T cells and reduced fibrosis. Additionally, there were fewer M2-like macrophages and an increase in mature DCs [43].

In the phase Ib/II PRINCE trial, sotigalimab (APX005M) was combined with gemcitabine plus nab-paclitaxel in patients with untreated metastatic PDAC. In the phase 1b portion, responses were observed in 14 of 24 (58%) DLT-evaluable patients [137]. In the randomized phase 2 portion (n=105), the primary endpoint of 1-year OS was met for the nivolumab/chemotherapy arm (57.7%, P=0.006 vs. 35% historical rate) but was not met for the sotigalimab/chemotherapy arm (48.1%, P=0.062) or the triple combination arm (41.3%, P=0.223). Multi-omic biomarker analyses identified distinct treatment-specific immune signature correlates. Survival was correlated with greater intratumoral $CD4^+$ T cell infiltration, increased circulating differentiated $CD4^+$ T cells, and increased APC numbers after sotigalimab/chemotherapy [138].

In OPTIMIZE-1, also a phase Ib/II study, CD40 agonist mitazalimab was combined with mFOLFIRINOX in previously untreated metastatic PDAC achieved a confirmed ORR of 40% (23/57 patients; updated confirmed ORR 42.1%), with a median PFS of 7.7 (95% CI: 5.8-11.3), median OS of 14.3 months (95% CI: 10.0-21.6), and a notably long median duration of response of 12.6 months. The 1-year PFS and OS rates were 34% (95% CI: 20.8-47.7) and 59% (95% CI: 44.2-71.1), respectively [136,139]. Here, objective responders displayed intratumoral myeloid and T cell activation, and mitazalimab-induced immune activation, including the expansion of effector $CD4^+$ T cells after the priming dose, correlated with improved outcomes. Furthermore, no cytokine release syndrome was reported with mitazalimab, in contrast to earlier-generation CD40 agonists, and the safety profile was consistent with mFOLFIRINOX alone, with no hepatotoxicity-related treatment discontinuations [136].

Across these early-phase studies, chemotherapy-CD40 combination strategies have demonstrated feasibility and acceptable safety, with no unexpected immune-related toxicities [136,137,140]. Observed cytokine release syndrome (grade 1-2 with selicrelumab and sotigalimab) and liver function test elevations were transient, dose-dependent, and clinically manageable. The most common grade ≥3 adverse events have been hematologic (neutropenia, anemia, thrombocytopenia), consistent with the chemotherapy backbone. These data collectively support the continued evaluation of chemotherapy and CD40 combination strategies in both metastatic disease and neoadjuvant settings, while underscoring that the chemotherapy choice, treatment sequencing, and timing of CD40 agonist administration relative to chemotherapy are likely key determinants of both efficacy and tolerability. The planned randomized, controlled, international phase III trial of mitazalimab with mFOLFIRINOX will be critical to establish whether the encouraging results from OPTIMIZE-1 translate into a survival benefit over standard chemotherapy alone [136,139].

4.3. CD40 Agonism Combined with Immune Checkpoint Inhibition

PD-1/PD-L1 and CTLA-4 blockade have limited efficacy in PDAC, in part due to inadequate baseline priming and dominant myeloid-driven suppression. CD40 agonism provides a mechanistic rationale to improve checkpoint responsiveness by licensing APCs, expanding tumor-specific T cell priming, and shifting macrophage programs away from suppressive phenotypes, thereby creating a microenvironment more permissive to checkpoint blockade [24,141,142]. Preclinical models support durable control and immune memory with CD40-ICI combinations [105,141,142]. CD40 agonist with immune checkpoint inhibition resulted in complete tumor regression in a therapy-resistant pancreatic cancer model [142]. This effect was not obtained with checkpoint inhibition alone. In another pancreatic cancer murine model, the combination of CD40 agonism and a checkpoint inhibitor has cured 63% of tumor-bearing animals and increased tumor-specific T cells in the pancreas [143]. These findings were confirmed in an orthotopic Kras/Trp53-mutant model, demonstrating that ICI and CD40 agonism combination significantly reduced the tumor burden and shifted the TME

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from a suppressive to an activated state, accompanied by enhanced DC migration and decreased immunosuppressive TAMs [105].

The phase 1b PRINCE trial clinically assessed sotigalimab (APX005M), a CD40 agonist, combined with gemcitabine and nab-paclitaxel, with or without the PD-1 inhibitor nivolumab, in patients with untreated metastatic PDAC. [137]. The treatment demonstrated feasibility with manageable safety and encouraging response activity (n=30; 24 DLT-evaluable). The observed cumulative ORR was 58% (14/24 DLT-evaluable patients), with a median PFS of 11.7 months (95% CI: 7.1-17.8), and median OS 20.1 months (95% CI: 10.5-not estimable). The cumulative 1-year PFS and OS rates were 44% (95% CI: 22.2-63.4) and 70% (95% CI: 46.9-84.3), respectively [137].

However, the subsequent randomized phase II portion of the trial (n=105) did not demonstrate a survival benefit for the CD40 treatment arms compared with chemotherapy alone [138]. The primary endpoint of 1-year OS was only met for patients receiving nivolumab plus chemotherapy (57.7% vs 35% historical rate, P = 0.006, n = 34), while the sotigalimab plus chemotherapy arm showed a modest but non-significant increase in the 1-year OS (48.1%, P = 0.062, n = 36). The triple combination arm with sotigalimab plus nivolumab plus chemotherapy did not meet the endpoint (41.3%, P = 0.223, n = 35). Despite no clear clinical benefit, survival after sotigalimab plus chemotherapy was correlated with greater $CD4^+$ T cell infiltration in the tumor and circulating differentiated $CD4^+$ T cells and APCs. Survival after nivolumab plus chemotherapy was correlated with a less immunosuppressive TME at baseline and a higher number of activated, antigen-experienced circulating T cells. Notably, no patient subset was identified for which the triple combination was more beneficial. The lack of survival benefit in this arm could potentially be explained by the observed signs of immune exhaustion [138].

CDX-1140, a fully human IgG2 CD40 agonist antibody designed to activate CD40 without requiring FcγR cross-linking, has also been utilized in combination strategies. A phase I trial (NCT03329950) evaluated CDX-1140 as monotherapy or in combination with CDX-301 (a recombinant human Fms-like tyrosine kinase 3 ligand (Flt3L)), pembrolizumab (anti-PD1), or gemcitabine/nab-paclitaxel in patients with advanced malignancies, including PDAC, and has been completed. Preliminary data were presented at conferences and indicated that CDX-1140 was well-tolerated with evidence of immune activation [144,145].

A separate randomized, open-label phase II trial (NCT04536077) evaluating CDX-1140 with or without CDX-301 in PDAC was terminated before completion. Although no peer-reviewed publication has been issued, some preliminary results have been shared [146]. In PDAC, low tissue levels of Flt3L partly contribute to the deficits in conventional DCs (cDCs). Combining systemic Flt3L with a CD40 agonist restored cDC numbers and function in both mouse models and samples from PDAC patients in clinical trials. However, when combined with Flt3L, dual therapy triggered a cDC-driven type I immune response characterized by T cell infiltration, IL-12 production, and reciprocal IFN-γ responses. cDC1s were responsible for $CD8^+$ T cell expansion, and T cell-derived IFN-γ enhanced cDC1 survival. However, the combination also increased regulatory T cells via cDC2 activation, thereby dampening immunity, highlighting the complexity of DC-centered approaches and the challenges of this combinatorial strategy.

A currently active phase I study (NCT04635995) is evaluating LVGN7409, a humanized monoclonal agonistic CD40 antibody, as monotherapy and in combination with anti-PD-1 and/or a CD137 agonist in patients with advanced or metastatic solid tumors, including potentially PDAC. Preliminary monotherapy data in 12 heavily pretreated patients did not show dose-limiting toxicities, and the treatment was well-tolerated. Among 9 evaluable patients, 44% achieved stable disease [147]. The combination arms incorporating checkpoint inhibition and CD137 agonism are of mechanistic interest, since CD137 (4-1BB) co-stimulation can amplify T cell effector function and survival downstream of CD40-licensed priming [148]. Results of this trial may inform whether this approach can overcome the immune suppression and limited T cell priming characteristics of PDAC.

Another phase Ib/II trial (NCT05419479) assessed a triple therapy of sotigalimab, domvanalimab (anti-TIGIT), and zimberelimab (anti-PD-1) in metastatic PDAC, even without checkpoint blockade.

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However, the trial is presently suspended, and no results have been published, underscoring the difficulties and complexity involved in multi-agent immunotherapy approaches for PDAC.

Collectively, these clinical trials demonstrate that overcoming PDACs' resistance to checkpoint blockade with CD40 agonism is more complex. Combining checkpoint blockade with CD40 agonism and chemotherapy or other regimens may be insufficient in unselected patients. Furthermore, layering the treatment regimen may paradoxically induce immune exhaustion rather than an additive benefit [138]. Understanding the drivers of immune exhaustion and finding ways to mitigate it [149] and biomarker-driven patient selection are significant determinants of treatment efficacy and improved survival outcomes.

4.4. Integration with Cancer Vaccines and Neoantigen-Directed Therapies

Vaccine strategies in PDAC, including personalized neoantigen approaches and KRAS-targeted vaccines, aim to generate de novo tumor-specific T cell responses against epitopes less constrained by tolerance [150–152]. CD40 agonism is central to DC maturation and antigen cross-presentation, and IL-12-associated Th1 programming, and may increase the magnitude and functional quality of vaccine-induced responses by enhancing antigen uptake, processing, and presentation [107,153]. This rationale was confirmed in a preclinical model, where CD40 and CD80/86 signaling in cDC1s was found to play a critical role in the effective antitumor immunity of a neoantigen-based therapeutic vaccine [154]. In a murine pancreatic cancer model, DC vaccination in combination with a CD40 agonist was necessary to improve survival in an advanced PDAC setting, whereas CD40 agonism alone was ineffective [107].

The phase I REACtiVe-2 evaluated DC vaccination with MesoPher (monocyte-derived DCs pulsed with an allogeneic tumor lysate) combined with CD40 agonist mitazalimab in patients with metastatic PDAC after completion of (m)FOLFIRINOX (n=16) [155]. A systemic increase in activated and vaccine-specific T cell responses was observed. In post-therapy tumor biopsies from metastatic sites, increased T cell infiltration and decreased collagen deposition were seen. Despite these immune changes, no objective radiological responses were recorded, and half of the patients (50%) had stable disease after three administrations. Notably, half of the patients in the study (50%) had progressive disease at baseline, indicating that halting the advancing tumor biology is challenging and suggesting that this treatment may not be effective for patients already experiencing disease progression at the time of study initiation. This underscores once more the importance of patient selection to ensure an optimal therapeutic response.

Another vaccination trial (NCT02600949) is exploring a personalized neoantigen peptide-based vaccine, administered alone or combined with imiquimod, pembrolizumab, and/or sotigalimab, in patients with advanced pancreatic and colorectal cancers. Although it is not a traditional vaccine, irreversible electroporation (IRE) serves as an in-situ antigen-release platform. As a non-thermal ablation method, IRE induces immunogenic cell death and releases tumor antigens [156]. A phase I trial (NCT06205849) is currently assessing the combination of surgical IRE with intratumoral mitazalimab injections in patients with locally advanced pancreatic cancer. This approach leverages the synergy between IRE-induced antigen release and CD40-driven APC licensing in a targeted manner and could inform future locoregional immunotherapy strategies for LAPC.

Overall, this data supports the idea that vaccine strategies and CD40 agonism address different bottlenecks in PDAC immunity. Vaccines supply specific antigen targets, while CD40 activation enhances APC licensing and activates costimulatory pathways necessary for effective T cell priming. Further research is needed to determine if combining these methods can overcome immune and stromal barriers, ultimately producing durable benefits across biomarker-defined patient groups.

4.5. Neoadjuvant and Window-of-Opportunity Experience

Window-of-opportunity and neoadjuvant studies offer a direct means to interrogate intratumoral pharmacodynamics and to test whether CD40 agonism can remodel the PDAC TME when immune trafficking and lymphatic drainage may be relatively more intact than in widely

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metastatic disease. Neoadjuvant selicrelumab has been reported to induce measurable immune activation and TME remodeling, supporting proof-of-mechanism in human PDAC, while the linkage between these pharmacodynamic effects and long-term clinical outcomes remains under active investigation [43]. These settings are also well-suited to optimize sequencing (e.g., relative timing to chemotherapy) and to validate tissue-based pharmacodynamic endpoints for subsequent trials.

4.6. Biomarkers: Prognostic Context and Predictors of Benefit

While pan-cancer analyses have linked higher CD40 expression to improved survival in some settings, this relationship is not uniform across tumor types, and the prognostic value appears limited in PDAC [30,157–161]. In PDAC transcriptomic analyses, *CD40* mRNA does not consistently function as an independent predictor of OS, and tumor-cell CD40 expression has not shown robust prognostic value in larger cohorts [30,162].

In a large multi-cohort study using spatial characterization of tumor samples from nine different solid tumors, CD40 expression was present in 68% of pancreatic adenocarcinomas [30]. However, CD40 expression on these tumor cells was not prognostic for OS. Similarly, in a pan-cancer transcriptomic analysis, high CD40 RNA expression was observed in 42% of pancreatic cancers and was correlated with improved OS in patients treated with immune checkpoint inhibitors, but not in multivariable analysis, suggesting that CD40 may not be an independent predictive biomarker [162]. In contrast to CD40 expression on tumor cells, serum levels of both soluble CD40 (sCD40) and CD40 ligand (sCD40L) were found to have some prognostic value. High sCD40 was associated with reduced OS, particularly in patients with neoadjuvant chemotherapy, and the combination with the tumor marker Carbohydrate Antigen 19.9 (CA19-9) increased its diagnostic value [163]. Similar to sCD40, soluble CD40 ligand (sCD40L) levels were also prognostic, with high levels associated with poor survival and correlated with unresectability and distant metastasis [117]. The predictive value of sCD40L was superior to that of the tumor markers CA19-9 and Carcinoembryonic Antigen (CEA).

Instead of CD40 expression, outcome associations in PDAC more strongly track with immune composition and functional state, particularly DC infiltration and macrophage polarization [43,138,139]. Neoadjuvant and adjuvant treatment with selicrelumab increased circulating CD4+ and CD8+ T cells and elevated inflammatory cytokine levels [43]. In the tumor, reduced fibrosis and decreased M2-like macrophages were observed, while an increase in mature DCs and enrichment of T cells were observed. Furthermore, systemic inflammatory cytokine levels were elevated after treatment. In the PRINCE trial, biomarker analysis revealed that higher baseline frequencies of circulating DCs, B cells, and experienced Th1 cells were associated with improved survival in patients treated with sotigalimab and gemcitabine plus nab-paclitaxel [138]. Within the tumor, Th1, Th2, and IFN-γ response signatures, and higher frequencies of tumor-infiltrating non-proliferating conventional and regulatory $CD4^+$ T cells, were associated with improved survival outcomes. In OPTIMIZE-1, baseline tumor-intrinsic gene signatures related to fibrosis and ECM remodeling were associated with improved survival after treatment with mitazalimab and mFOLFIRINOX [139]. In peripheral blood, increased activation and proliferation of circulating T cells and NK cells were observed.

Collectively, these data support prioritizing on-treatment pharmacodynamic biomarkers, such as APC activation, shifts in myeloid polarization, T cell activation and recruitment, and immune infiltration and stromal remodeling in the TME, as more plausible indicators of effective pathway engagement over baseline CD40 abundance alone.

4.7. Safety Considerations and Toxicity Management

CD40 agonists can induce systemic immune activation with cytokine-associated symptoms, transient cytopenias, and liver enzyme elevations; cytokine release syndrome is typically moderate and transient, consistent with activation of immune and vascular compartments [24,36,43,98,164,165]. Transient transaminase elevations are often detected within around 24 hours of dosing and may persist for weeks before resolving, emphasizing the need for laboratory monitoring and dosing

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strategies that balance potency with tolerability [36,43,98,164,165]. Safety profiles vary among agents, plausibly reflecting differences in FcγR engagement, clustering requirements, and schedule, and intravenous administration has generally been feasible in early-phase studies, while optimal regimens continue to be refined. In PDAC combination studies, including DC vaccination plus CD40 agonism in the REACtiVe-2 trial, overall tolerability has been reported as manageable, supporting further development while underscoring the need to mitigate toxicity and optimize regimens to realize therapeutic benefit [43,136–138,140,155].

5. Challenges and Future Directions

5.1. Limitation to Consistent Clinical Benefit

Despite compelling biology, translating CD40 agonism into durable efficacy in PDAC remains difficult. First, PDAC imposes strong baseline constraints, marked myeloid dominance, limited endogenous priming, dense desmoplasia, and abnormal vasculature, which can blunt effector trafficking and function even when APC activation is achieved [4,104]. Second, CD40 agonists are not interchangeable: differences in clustering requirements, FcγR dependence, and exposure profiles likely contribute to heterogeneous pharmacodynamics and safety across agents [131,166]. Third, antitumor activity appears highly context dependent, with outcomes shaped by disease setting (metastatic vs neoadjuvant), tumor burden, immune composition, and treatment sequencing [43,82,136–138]. Finally, adaptive counter-regulation, including re-emergence of suppressive myeloid programs, regulatory T cell expansion, compensatory inhibitory pathways, and stromal reconstitution, may limit durability and argue for longitudinal tissue-based interrogation in trials [130,143,146,167].

5.1. Biomarkers and Patient Selection: Beyond Baseline CD40 Abundance

Available data do not support baseline CD40 expression as a reliable prognostic or predictive biomarker in PDAC [30,162]. Future stratification is more likely to benefit from biomarkers that capture the functional state and spatial organization of the microenvironment, such as DC subset abundance and activation [143,146,168], macrophage polarization states [130], TLS features [169,170], and stromal programs [139], rather than single-marker expression. Integrating high-dimensional profiling (single-cell approaches, multiplex tissue imaging, spatial transcriptomics/proteomics, and longitudinal immune monitoring) should help define tumor-immune states permissive to CD40-driven priming and identify early pharmacodynamic signals associated with benefit [171–173]. In parallel, emphasis should shift toward on-treatment pharmacodynamic endpoints (APC activation signatures, myeloid reprogramming, chemokine programs linked to trafficking, and quantitative immune infiltration/stromal remodeling readouts) as practical markers of effective pathway engagement [43,82,139].

5.2. Optimization of Dosing, Scheduling, and Delivery

The therapeutic window of CD40 agonists remains a central development constraint, requiring dosing strategies that maximize intratumoral pharmacodynamics while limiting systemic inflammatory toxicity [174]. Step-up dosing, intermittent schedules, and combination-dependent dose modulation warrant systematic evaluation, ideally linked to mechanistic readouts (e.g., APC activation and cytokine programs) that can define an exposure-response relationship [136,165]. Alternative delivery approaches, including locoregional or intratumoral administration, may improve spatial specificity and reduce systemic exposure [35,175], but will require careful feasibility evaluation in PDAC and validation that local activity translates into systemic antitumor immunity [58,176].

5.3. Context-Dependent Tumor-Intrinsic CD40 Signaling

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An unresolved translational issue is tumor-intrinsic CD40 signaling, which can produce divergent outcomes depending on tumor state and microenvironmental cues, with NF-κB/MAPK programs linked to either apoptosis or pro-survival/proliferative effects in different settings [29,124,125]. Given that CD40-targeted therapies may engage tumor-cell CD40 in a subset of PDACs [30,162], future work should clarify how genomic alterations, epigenetic states, and extrinsic cytokine signals shape tumor-intrinsic CD40 responses [103,177,178], and whether tumor-cell CD40 status should inform patient selection or combination strategy to avoid unintended protumor signaling [33,40].

5.4. Next-Generation CD40 Agonists and Rational Combinations

Next-generation formats, including Fc-engineered antibodies [179,180], multivalent ligand-mimetic constructs [181], bispecific/conditional agonists [33,176,182,183], and nanoparticle or other tumor-localizing delivery strategies [58,175,184], aim to enhance productive receptor clustering in the relevant immune niche while minimizing systemic activation. Mechanistically, CD40 agonism is unlikely to provide durable benefit as monotherapy and is best developed within multidimensional regimens that coordinate antigen supply with APC licensing and relieve downstream suppression [43,107,136,146]. Chemotherapy and radiotherapy remain important partners for antigen release and microenvironmental modulation [136,137,185], checkpoint blockade may be most effective when paired with demonstrable priming [105,138,142,186], and vaccine/DC/neoantigen strategies provide a direct route to increase antigen-focused T cell responses [107,155]; collectively, these combinations are likely to be particularly informative in neoadjuvant or minimal residual disease settings where tumor burden is lower and immune trafficking may be less constrained [43,107].

5.5. Standardization of CD40 Measurement and Reporting

To enable meaningful comparison across studies and support biomarker development, CD40 measurement requires greater standardization. Bulk transcriptomic readouts quantify *CD40* mRNA in mixed tissue and are sensitive to immune/stromal admixture [162,187,188], while spatial profiling confirms multicellular heterogeneity and indicates that treatment exposure can shift compartmental states, altering bulk signals independent of true cell-intrinsic changes [172,189]. Protein-level assays (IHC, multiplex IF, quantitative immunofluorescence) improve compartment attribution but vary by antibody clone, platform, and scoring method and remain sensitive to sampling and intratumoral heterogeneity [121,190]. Accordingly, studies should explicitly report assay modality, scored compartment(s), scoring thresholds, specimen type and disease site, and treatment context, and where feasible, integrate bulk and spatially resolved approaches to support reproducible interpretation [172,173,189,191,192].

6. Conclusion

CD40 is a multicompartment immunotherapeutic target in PDAC, with the capacity to license antigen-presenting cells, reprogram suppressive myeloid states, and contribute to stromal and vascular remodeling, which may help relieve immune exclusion. Early clinical studies have shown clear pharmacodynamic immune engagement, but clinical benefit has remained inconsistent, and baseline CD40 expression alone appears insufficient to guide prognosis or therapeutic selection. Further progress will likely depend on selecting CD40 agonist modalities with appropriate clustering and FcγR-dependent properties, optimizing treatment sequencing to align tumor antigen availability with APC licensing and effector T-cell support, prioritizing on-treatment pharmacodynamic and spatial biomarkers over static expression measures, and improving safety through refined dosing, scheduling, and potentially tumor-localized or conditionally active agonist designs.

A particularly interesting direction is the combination of CD40 agonism with next-generation cancer vaccines, including mRNA-based, KRAS-targeted, neoantigen-directed, and dendritic cell–based platforms. In this setting, CD40 activation may provide the APC licensing and myeloid

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reprogramming required to convert vaccine-induced antigen recognition into more effective and durable T-cell priming within the hostile PDAC microenvironment. Although early preclinical and clinical data support this rationale, dedicated studies are needed to define optimal timing, combination partners, biomarker strategies, and patient selection. With continued refinement, CD40-directed approaches remain a biologically plausible strategy to increase the proportion of patients with PDAC who achieve meaningful and durable immune-mediated tumor control.

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References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. May-Jun 2024;74(3):229-263.
2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res*. Jun 1 2014;74(11):2913-21. doi:0008-5472.CAN-14-0155 [pii]
3. 10.1158/0008-5472.CAN-14-0155
4. Stoop TF, Javed AA, Oba A, et al. Pancreatic cancer. *Lancet*. Apr 5 2025;405(10485):1182-1202.
5. Park W, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. *Jama*. Sep 7 2021;326(9):851-862.
6. Siegel RL, Kratzer TB, Wagle NS, Sung H, Jemal A. Cancer statistics, 2026. *CA Cancer J Clin*. Jan-Feb 2026;76(1):e70043.
7. André T, Elez E, Lenz HJ, et al. Nivolumab plus ipilimumab versus nivolumab in microsatellite instability-high metastatic colorectal cancer (CheckMate 8HW): a randomised, open-label, phase 3 trial. *Lancet*. Feb 1 2025;405(10476):383-395.
8. Chalabi M, Fanchi LF, Dijkstra KK, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med*. Apr 2020;26(4):566-576.
9. Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet*. May 4 2019;393(10183):1819-1830.
10. Spicer JD, Garassino MC, Wakelee H, et al. Neoadjuvant pembrolizumab plus chemotherapy followed by adjuvant pembrolizumab compared with neoadjuvant chemotherapy alone in patients with early-stage non-small-cell lung cancer (KEYNOTE-671): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. Sep 28 2024;404(10459):1240-1252.
11. Wolchok JD, Chiarion-Sileni V, Rutkowski P, et al. Final, 10-Year Outcomes with Nivolumab plus Ipilimumab in Advanced Melanoma. *N Engl J Med*. Jan 2 2025;392(1):11-22.
12. Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet*. Jul 3 2021;398(10294):27-40.
13. Tannir NM, Albigès L, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 8-year follow-up results of efficacy and safety from the phase III CheckMate 214 trial. *Ann Oncol*. Nov 2024;35(11):1026-1038.
14. Abou-Alfa GK, Lau G, Kudo M, et al. Tremelimumab plus Durvalumab in Unresectable Hepatocellular Carcinoma. *NEJM Evid*. Aug 2022;1(8):EVIDoa2100070.
15. Powles T, Valderrama BP, Gupta S, et al. Enfortumab Vedotin and Pembrolizumab in Untreated Advanced Urothelial Cancer. *N Engl J Med*. Mar 7 2024;390(10):875-888.

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16. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. Jun 28 2012;366(26):2455-65.
17. Renouf DJ, Loree JM, Knox JJ, et al. The CCTG PA.7 phase II trial of gemcitabine and nab-paclitaxel with or without durvalumab and tremelimumab as initial therapy in metastatic pancreatic ductal adenocarcinoma. *Nat Commun*. Aug 26 2022;13(1):5020.
18. O'Reilly EM, Oh DY, Dhani N, et al. Durvalumab With or Without Tremelimumab for Patients With Metastatic Pancreatic Ductal Adenocarcinoma: A Phase 2 Randomized Clinical Trial. *JAMA Oncol*. Oct 1 2019;5(10):1431-1438.
19. Ho WJ, Jaffee EM, Zheng L. The tumour microenvironment in pancreatic cancer - clinical challenges and opportunities. *Nat Rev Clin Oncol*. Sep 2020;17(9):527-540. doi:10.1038/s41571-020-0363-5
20. Murakami T, Hiroshima Y, Matsuyama R, Homma Y, Hoffman RM, Endo I. Role of the tumor microenvironment in pancreatic cancer. *Ann Gastroenterol Surg*. Mar 2019;3(2):130-137.
21. Dallal RM, Christakos P, Lee K, Egawa S, Son YI, Lotze MT. Paucity of dendritic cells in pancreatic cancer. *Surgery*. Feb 2002;131(2):135-8.
22. Hegde S, Krisnawan VE, Herzog BH, et al. Dendritic Cell Paucity Leads to Dysfunctional Immune Surveillance in Pancreatic Cancer. *Cancer Cell*. Mar 16 2020;37(3):289-307 e9.
23. Orhan A, Vogelsang RP, Andersen MB, et al. The prognostic value of tumour-infiltrating lymphocytes in pancreatic cancer: a systematic review and meta-analysis. *Eur J Cancer*. Jun 2020;132:71-84.
24. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*. Jun 4 1998;393(6684):480-3.
25. Vonderheide RH. CD40 Agonist Antibodies in Cancer Immunotherapy. *Annu Rev Med*. Jan 27 2020;71:47-58.
26. Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. *Annu Rev Immunol*. 1998;16:111-35.
27. van Kooten C, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol*. Jan 2000;67(1):2-17.
28. Vonderheide RH. Prospect of targeting the CD40 pathway for cancer therapy. *Clin Cancer Res*. Feb 15 2007;13(4):1083-8.
29. Caux C, Massacrier C, Vanbervliet B, et al. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med*. Oct 1 1994;180(4):1263-72.
30. He S, Zhao H, Fei M, et al. Expression of the co-signaling molecules CD40-CD40L and their growth inhibitory effect on pancreatic cancer in vitro. *Oncol Rep*. Jul 2012;28(1):262-8.
31. Bates KM, Vathiotis I, MacNeil T, et al. Spatial characterization and quantification of CD40 expression across cancer types. *BMC Cancer*. Mar 9 2023;23(1):220.
32. Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. Mar 25 2011;331(6024):1612-6.
33. Long KB, Gladney WL, Tooker GM, Graham K, Fraietta JA, Beatty GL. IFNγ and CCL2 Cooperate to Redirect Tumor-Infiltrating Monocytes to Degrade Fibrosis and Enhance Chemotherapy Efficacy in Pancreatic Carcinoma. *Cancer Discov*. Apr 2016;6(4):400-413.
34. McVey JC, Beatty GL. Facts and Hopes of CD40 Agonists in Cancer Immunotherapy. *Clin Cancer Res*. Jun 3 2025;31(11):2079-2087.
35. Mangsbo SM, Broos S, Fletcher E, et al. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. *Clin Cancer Res*. Mar 1 2015;21(5):1115-26.
36. Irenaeus SMM, Nielsen D, Ellmark P, et al. First-in-human study with intratumoral administration of a CD40 agonistic antibody, ADC-1013, in advanced solid malignancies. *Int J Cancer*. Sep 1 2019;145(5):1189-1199.
37. Moreno V, Perets R, Peretz-Yablonski T, et al. A phase 1 study of intravenous mitazalimab, a CD40 agonistic monoclonal antibody, in patients with advanced solid tumors. *Invest New Drugs*. Feb 2023;41(1):93-104.
38. Baxendale AJ, Dawson CW, Stewart SE, et al. Constitutive activation of the CD40 pathway promotes cell transformation and neoplastic growth. *Oncogene*. Nov 24 2005;24(53):7913-23.
39. Murugaiyan G, Martin S, Saha B. CD40-induced countercurrent conduits for tumor escape or elimination? *Trends Immunol*. Nov 2007;28(11):467-73.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 May 2026 doi:10.20944/preprints202605.0143.v1

40. Korniluk A, Kemona H, Dymicka-Piekarska V. Multifunctional CD40L: pro- and anti-neoplastic activity. *Tumour Biol*. Oct 2014;35(10):9447-57.
41. Batrla R, Linnebacher M, Rudy W, Stumm S, Wallwiener D, Gückel B. CD40-expressing carcinoma cells induce down-regulation of CD40 ligand (CD154) and impair T-cell functions. *Cancer Res*. Apr 1 2002;62(7):2052-7.
42. Khaliq AM, Rajamohan M, Saeed O, et al. Spatial transcriptomic analysis of primary and metastatic pancreatic cancers highlights tumor microenvironmental heterogeneity. *Nat Genet*. Nov 2024;56(11):2455-2465.
43. Gong R, Chen Y, Li C, Zhang H, Liu Z, Yu Q. Targeting myeloid cells in pancreatic ductal adenocarcinoma: from primary tumors to liver metastasis. *Front Immunol*. 2025;16:1555036.
44. Byrne KT, Betts CB, Mick R, et al. Neoadjuvant Selicrelumab, an Agonist CD40 Antibody, Induces Changes in the Tumor Microenvironment in Patients with Resectable Pancreatic Cancer. *Clin Cancer Res*. Aug 15 2021;27(16):4574-4586.
45. Ohlund D, Handly-Santana A, Biffi G, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med*. Mar 6 2017;214(3):579-596. doi:jem.20162024 [pii]
46. 20162024 [pii]
47. 10.1084/jem.20162024
48. Rebelo R, Xavier CPR, Giovannetti E, Vasconcelos MH. Fibroblasts in pancreatic cancer: molecular and clinical perspectives. *Trends Mol Med*. Jun 2023;29(6):439-453.
49. Karmann K, Hughes CC, Schechner J, Fanslow WC, Pober JS. CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression. *Proc Natl Acad Sci U S A*. May 9 1995;92(10):4342-6.
50. Urban D, Thanabalasingam U, Stibenz D, et al. CD40/CD40L interaction induces E-selectin dependent leukocyte adhesion to human endothelial cells and inhibits endothelial cell migration. *Biochem Biophys Res Commun*. Jan 7 2011;404(1):448-52.
51. Shintani Y, Fujiwara A, Kimura T, et al. IL-6 Secreted from Cancer-Associated Fibroblasts Mediates Chemoresistance in NSCLC by Increasing Epithelial-Mesenchymal Transition Signaling. *J Thorac Oncol*. Sep 2016;11(9):1482-92.
52. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. *Oncogene*. Mar 30 2017;36(13):1770-1778.
53. Chakravarthy A, Khan L, Bensler NP, Bose P, De Carvalho DD. TGF-β-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nat Commun*. Nov 8 2018;9(1):4692.
54. Grout JA, Sirven P, Leader AM, et al. Spatial Positioning and Matrix Programs of Cancer-Associated Fibroblasts Promote T-cell Exclusion in Human Lung Tumors. *Cancer Discov*. Nov 2 2022;12(11):2606-2625.
55. Andersson H, Nyesiga B, Hermodsson T, et al. Next-generation CD40 agonists for cancer immunotherapy. *Expert Opin Biol Ther*. May 2024;24(5):351-363.
56. Bellone G, Carbone A, Smirne C, et al. Cooperative induction of a tolerogenic dendritic cell phenotype by cytokines secreted by pancreatic carcinoma cells. *J Immunol*. Sep 1 2006;177(5):3448-60.
57. Xie Z, Fang Y, Zhang X, et al. The emerging role of dendritic cells in the tumor microenvironment: from antigen presentation to targeted immunotherapy. *Cell Death Dis*. Dec 22 2025;16(1):900.
58. Zuo C, Baer JM, Knolhoff BL, et al. Stromal and therapy-induced macrophage proliferation promotes PDAC progression and susceptibility to innate immunotherapy. *J Exp Med*. Jun 5 2023;220(6)doi:213966 [pii]
59. jem.20212062 [pii]
60. 10.1084/jem.20212062
61. Lehmann J, Thelen M, Kreer C, et al. Tertiary Lymphoid Structures in Pancreatic Cancer are Structurally Homologous, Share Gene Expression Patterns and B-cell Clones with Secondary Lymphoid Organs, but Show Increased T-cell Activation. *Cancer Immunol Res*. Mar 4 2025;13(3):323-336.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 May 2026 doi:10.20944/preprints202605.0143.v1

62. Pan PY, Ma G, Weber KJ, et al. Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res*. Jan 1 2010;70(1):99-108.
63. Minaei E, Wade SJ, Penney C, et al. Localized Delivery of Anti-CD40/PD-1 Immunotherapy via a Polymeric Implant is Efficacious, Remodels the Tumor Immune Microenvironment and Improves Safety in a PDAC Mouse Model. *Adv Healthc Mater*. Feb 2026;15(6):e03780.
64. Baumann D, Hägele T, Mochayedi J, et al. Proimmunogenic impact of MEK inhibition synergizes with agonist anti-CD40 immunostimulatory antibodies in tumor therapy. *Nat Commun*. May 1 2020;11(1):2176.
65. Carbone E, Ruggiero G, Terrazzano G, et al. A new mechanism of NK cell cytotoxicity activation: the CD40-CD40 ligand interaction. *J Exp Med*. Jun 16 1997;185(12):2053-60.
66. Terrazzano G, Pisanti S, Grimaldi S, et al. Interaction between natural killer and dendritic cells: the role of CD40, CD80 and major histocompatibility complex class i molecules in cytotoxicity induction and interferon-gamma production. *Scand J Immunol*. Apr 2004;59(4):356-62.
67. Jyothi MD, Khar A. Regulation of CD40L expression on natural killer cells by interleukin-12 and interferon gamma: its role in the elicitation of an effective antitumor immune response. *Cancer Immunol Immunother*. Dec 2000;49(10):563-72.
68. Turner JG, Rakhmilevich AL, Burdelya L, et al. Anti-CD40 antibody induces antitumor and antimetastatic effects: the role of NK cells. *J Immunol*. Jan 1 2001;166(1):89-94.
69. Tsukamoto N, Kobayashi N, Azuma S, Yamamoto T, Inoue J. Two differently regulated nuclear factor kappaB activation pathways triggered by the cytoplasmic tail of CD40. *Proc Natl Acad Sci U S A*. Feb 16 1999;96(4):1234-9.
70. Foight GW, Keating AE. Comparison of the peptide binding preferences of three closely related TRAF paralogs: TRAF2, TRAF3, and TRAF5. *Protein Sci*. Jul 2016;25(7):1273-89.
71. Ma Y, Ding J, Chen Y. The effect of CD40 agonist antibody therapy on the pancreatic cancer microenvironment. *Naunyn Schmiedebergs Arch Pharmacol*. Jan 2026;399(2):1897-1915.
72. Sun SC. Non-canonical NF-κB signaling pathway. *Cell Res*. Jan 2011;21(1):71-85.
73. Clatza A, Bonifaz LC, Vignali DA, Moreno J. CD40-induced aggregation of MHC class II and CD80 on the cell surface leads to an early enhancement in antigen presentation. *J Immunol*. Dec 15 2003;171(12):6478-87.
74. Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med*. Aug 1 1996;184(2):747-52.
75. Gallagher E, Enzler T, Matsuzawa A, et al. Kinase MEKK1 is required for CD40-dependent activation of the kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production. *Nat Immunol*. Jan 2007;8(1):57-63.
76. Tang T, Cheng X, Truong B, Sun L, Yang X, Wang H. Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint. *Pharmacol Ther*. Mar 2021;219:107709.
77. Yamato I, Sho M, Nomi T, et al. Clinical importance of B7-H3 expression in human pancreatic cancer. *Br J Cancer*. Nov 17 2009;101(10):1709-16.
78. Zhao X, Li DC, Zhu XG, et al. B7-H3 overexpression in pancreatic cancer promotes tumor progression. *Int J Mol Med*. Feb 2013;31(2):283-91.
79. Inamura K, Takazawa Y, Inoue Y, et al. Tumor B7-H3 (CD276) Expression and Survival in Pancreatic Cancer. *J Clin Med*. Jul 10 2018;7(7)
80. Ma Y, Yang Y, Huang Y, et al. A B7H3-targeting antibody-drug conjugate in advanced solid tumors: a phase 1/1b trial. *Nat Med*. Jun 2025;31(6):1949-1957.
81. Duan J, Sun Y, Wang Q, et al. HS-20093, a B7-H3-targeted antibody-drug conjugate in lung cancer: Results from the ARTEMIS-001 phase 1a/b trial. *Cancer Cell*. Apr 13 2026;44(4):846-857 e3.
82. Hostager BS, Bishop GA. CD40-Mediated Activation of the NF-κB2 Pathway. *Front Immunol*. Nov 15 2013;4:376.
83. Zhou Y, Richmond A, Yan C. Harnessing the potential of CD40 agonism in cancer therapy. *Cytokine Growth Factor Rev*. Feb 2024;75:40-56.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 May 2026 doi:10.20944/preprints202605.0143.v1

84. Hanissian SH, Geha RS. Jak3 is associated with CD40 and is critical for CD40 induction of gene expression in B cells. *Immunity*. Apr 1997;6(4):379-87.
85. Revy P, Hivroz C, Andreu G, et al. Activation of the Janus kinase 3-STAT5a pathway after CD40 triggering of human monocytes but not of resting B cells. *J Immunol*. Jul 15 1999;163(2):787-93.
86. Säemann MD, Diakos C, Kelemen P, et al. Prevention of CD40-triggered dendritic cell maturation and induction of T-cell hyporeactivity by targeting of Janus kinase 3. *Am J Transplant*. Nov 2003;3(11):1341-9.
87. Wattenberg MM, Herrera VM, Giannone MA, Gladney WL, Carpenter EL, Beatty GL. Systemic inflammation is a determinant of outcomes of CD40 agonist-based therapy in pancreatic cancer patients. *JCI Insight*. Mar 8 2021;6(5)
88. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer*. Nov 2009;9(11):798-809.
89. Lesina M, Kurkowski MU, Ludes K, et al. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell*. Apr 12 2011;19(4):456-69.
90. Corcoran RB, Contino G, Deshpande V, et al. STAT3 plays a critical role in KRAS-induced pancreatic tumorigenesis. *Cancer Res*. Jul 15 2011;71(14):5020-9.
91. Fukuda A, Wang SC, Morris JPt, et al. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*. Apr 12 2011;19(4):441-55.
92. Li H, Huang C, Huang K, et al. STAT3 knockdown reduces pancreatic cancer cell invasiveness and matrix metalloproteinase-7 expression in nude mice. *PLoS One*. 2011;6(10):e25941.
93. Chang Q, Bournazou E, Sansone P, et al. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. *Neoplasia*. Jul 2013;15(7):848-62.
94. Scholz A, Heinze S, Detjen KM, et al. Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. *Gastroenterology*. Sep 2003;125(3):891-905.
95. Wei D, Le X, Zheng L, et al. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene*. Jan 23 2003;22(3):319-29.
96. Hurwitz HI, Uppal N, Wagner SA, et al. Randomized, Double-Blind, Phase II Study of Ruxolitinib or Placebo in Combination With Capecitabine in Patients With Metastatic Pancreatic Cancer for Whom Therapy With Gemcitabine Has Failed. *J Clin Oncol*. Dec 1 2015;33(34):4039-47.
97. Hurwitz H, Van Cutsem E, Bendell J, et al. Ruxolitinib + capecitabine in advanced/metastatic pancreatic cancer after disease progression/intolerance to first-line therapy: JANUS 1 and 2 randomized phase III studies. *Invest New Drugs*. Aug 2018;36(4):683-695.
98. Beatty GL, Shahda S, Beck T, et al. A Phase Ib/II Study of the JAK1 Inhibitor, Itacitinib, plus nab-Paclitaxel and Gemcitabine in Advanced Solid Tumors. *Oncologist*. Jan 2019;24(1):14-e10.
99. Parampalli Yajnanarayana S, Stübig T, Cornez I, et al. JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. *Br J Haematol*. Jun 2015;169(6):824-33.
100. Klein K, Stoiber D, Sexl V, Witalisz-Siepracka A. Untwining Anti-Tumor and Immunosuppressive Effects of JAK Inhibitors-A Strategy for Hematological Malignancies? *Cancers (Basel)*. May 26 2021;13(11)
101. Kubo S, Yamaoka K, Kondo M, et al. The JAK inhibitor, tofacitinib, reduces the T cell stimulatory capacity of human monocyte-derived dendritic cells. *Ann Rheum Dis*. Dec 2014;73(12):2192-8.
102. Chand Dakal T, Dhabhai B, Agarwal D, et al. Mechanistic basis of co-stimulatory CD40-CD40L ligation mediated regulation of immune responses in cancer and autoimmune disorders. *Immunobiology*. Mar 2020;225(2):151899.
103. Johnson P, Challis R, Chowdhury F, et al. Clinical and biological effects of an agonist anti-CD40 antibody: a Cancer Research UK phase I study. *Clin Cancer Res*. Mar 15 2015;21(6):1321-8.
104. Ino Y, Yamazaki-Itoh R, Shimada K, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br J Cancer*. Mar 5 2013;108(4):914-23.
105. Hou YC, Chao YJ, Hsieh MH, Tung HL, Wang HC, Shan YS. Low $CD8^+$ T Cell Infiltration and High PD-L1 Expression Are Associated with Level of $CD44^+/CD133^+$ Cancer Stem Cells and Predict an Unfavorable Prognosis in Pancreatic Cancer. *Cancers (Basel)*. Apr 15 2019;11(4)

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 May 2026 doi:10.20944/preprints202605.0143.v1

106. Fukunaga A, Miyamoto M, Cho Y, et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas*. Jan 2004;28(1):e26-31.
107. Garcia Garcia CJ, Huang Y, Fuentes NR, et al. Stromal HIF2 Regulates Immune Suppression in the Pancreatic Cancer Microenvironment. *Gastroenterology*. Jun 2022;162(7):2018-2031.
108. Caronni N, La Terza F, Vittoria FM, et al. IL-1β(+) macrophages fuel pathogenic inflammation in pancreatic cancer. *Nature*. Nov 2023;623(7986):415-422.
109. Shakiba M, Tuveson DA. Macrophages and fibroblasts as regulators of the immune response in pancreatic cancer. *Nat Immunol*. May 2025;26(5):678-691.
110. Ichikawa J, Okuda H, Kawano K, et al. A Combinatorial Effect of Immune Checkpoint Inhibitors and CD40 Agonistic Antibody in Murine Pancreatic Cancer Model. *Cancer Sci*. Jan 2026;117(1):8-18.
111. Byrne KT, Vonderheide RH. CD40 Stimulation Obviates Innate Sensors and Drives T Cell Immunity in Cancer. *Cell Rep*. Jun 21 2016;15(12):2719-32.
112. Lau SP, van Montfoort N, Kinderman P, et al. Dendritic cell vaccination and CD40-agonist combination therapy licenses T cell-dependent antitumor immunity in a pancreatic carcinoma murine model. *J Immunother Cancer*. Jul 2020;8(2)doi:jitc-2020-000772 [pii]
113. 10.1136/jitc-2020-000772
114. Wagner AH, Gebauer M, Pollok-Kopp B, Hecker M. Cytokine-inducible CD40 expression in human endothelial cells is mediated by interferon regulatory factor-1. *Blood*. Jan 15 2002;99(2):520-5.
115. Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. *J Am Coll Cardiol*. Aug 18 2009;54(8):669-77.
116. Vuong JT, Stein-Merlob AF, Nayeri A, Sallam T, Neilan TG, Yang EH. Immune Checkpoint Therapies and Atherosclerosis: Mechanisms and Clinical Implications: JACC State-of-the-Art Review. *J Am Coll Cardiol*. Feb 15 2022;79(6):577-593.
117. Prontera C, Martelli N, Evangelista V, et al. Homocysteine modulates the CD40/CD40L system. *J Am Coll Cardiol*. Jun 5 2007;49(22):2182-90.
118. Mach F, Schönbeck U, Fabunmi RP, et al. T lymphocytes induce endothelial cell matrix metalloproteinase expression by a CD40L-dependent mechanism: implications for tubule formation. *Am J Pathol*. Jan 1999;154(1):229-38.
119. Eriksson E, Moreno R, Milenova I, et al. Activation of myeloid and endothelial cells by CD40L gene therapy supports T-cell expansion and migration into the tumor microenvironment. *Gene Ther*. Feb 2017;24(2):92-103.
120. Luheshi NM, Coates-Ulrichsen J, Harper J, et al. Transformation of the tumour microenvironment by a CD40 agonist antibody correlates with improved responses to PD-L1 blockade in a mouse orthotopic pancreatic tumour model. *Oncotarget*. Apr 5 2016;7(14):18508-20.
121. Melter M, Reinders ME, Sho M, et al. Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. *Blood*. Dec 1 2000;96(12):3801-8.
122. Reinders ME, Sho M, Robertson SW, Geehan CS, Briscoe DM. Proangiogenic function of CD40 ligand-CD40 interactions. *J Immunol*. Aug 1 2003;171(3):1534-41.
123. Chung HW, Lim JB. Clinical significance of elevated serum soluble CD40 ligand levels as a diagnostic and prognostic tumor marker for pancreatic ductal adenocarcinoma. *J Transl Med*. Apr 21 2014;12:102.
124. Flaxenburg JA, Melter M, Lapchak PH, Briscoe DM, Pal S. The CD40-induced signaling pathway in endothelial cells resulting in the overexpression of vascular endothelial growth factor involves Ras and phosphatidylinositol 3-kinase. *J Immunol*. Jun 15 2004;172(12):7503-9.
125. Selvaraj S, Raundhal M, Patidar A, Saha B. Anti-VEGF antibody enhances the antitumor effect of CD40. *Int J Cancer*. Oct 15 2014;135(8):1983-8.
126. Kashyap AS, Schmittnaegel M, Rigamonti N, et al. Optimized antiangiogenic reprogramming of the tumor microenvironment potentiates CD40 immunotherapy. *Proc Natl Acad Sci U S A*. Jan 7 2020;117(1):541-551.
127. Shoji Y, Miyamoto M, Ishikawa K, et al. The CD40-CD154 interaction would correlate with proliferation and immune escape in pancreatic ductal adenocarcinoma. *J Surg Oncol*. Mar 1 2011;103(3):230-8.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 May 2026 doi:10.20944/preprints202605.0143.v1

128. Alexandroff AB, Jackson AM, Paterson T, et al. Role for CD40-CD40 ligand interactions in the immune response to solid tumours. *Mol Immunol*. Jun 2000;37(9):515-26.
129. Schiele P, Japp AS, Stark R, et al. CD8(+) T cell-derived CD40L mediates noncanonical cytotoxicity in CD40-expressing cancer cells. *Sci Adv*. May 23 2025;11(21):eadr9331.
130. Davies CC, Mason J, Wakelam MJ, Young LS, Eliopoulos AG. Inhibition of phosphatidylinositol 3-kinase- and ERK MAPK-regulated protein synthesis reveals the pro-apoptotic properties of CD40 ligation in carcinoma cells. *J Biol Chem*. Jan 9 2004;279(2):1010-9.
131. Elmetwali T, Young LS, Palmer DH. CD40 ligand-induced carcinoma cell death: a balance between activation of TNFR-associated factor (TRAF) 3-dependent death signals and suppression of TRAF6-dependent survival signals. *J Immunol*. Jan 15 2010;184(2):1111-20.
132. Li D, Zhong Y, Zhou Y, et al. Autocrine TNF-α-mediated NF-κB activation is a determinant for evasion of CD40-induced cytotoxicity in cancer cells. *Biochem Biophys Res Commun*. Jul 5 2013;436(3):467-72.
133. Ahmed-Choudhury J, Russell CL, Randhawa S, Young LS, Adams DH, Afford SC. Differential induction of nuclear factor-kappaB and activator protein-1 activity after CD40 ligation is associated with primary human hepatocyte apoptosis or intrahepatic endothelial cell proliferation. *Mol Biol Cell*. Apr 2003;14(4):1334-45.
134. Fonsatti E, Maio M, Altomonte M, Hersey P. Biology and clinical applications of CD40 in cancer treatment. *Semin Oncol*. Oct 2010;37(5):517-23.
135. Murugaiyan G, Agrawal R, Mishra GC, Mitra D, Saha B. Differential CD40/CD40L expression results in counteracting antitumor immune responses. *J Immunol*. Feb 15 2007;178(4):2047-55.
136. Murgaski A, Kiss M, Van Damme H, et al. Efficacy of CD40 Agonists Is Mediated by Distinct cDC Subsets and Subverted by Suppressive Macrophages. *Cancer Res*. Oct 17 2022;82(20):3785-3801.
137. Dahan R, Barnhart BC, Li F, Yamniuk AP, Korman AJ, Ravetch JV. Therapeutic Activity of Agonistic, Human Anti-CD40 Monoclonal Antibodies Requires Selective FcγR Engagement. *Cancer Cell*. Jun 13 2016;29(6):820-831.
138. Beatty GL, Winograd R, Evans RA, et al. Exclusion of T Cells From Pancreatic Carcinomas in Mice Is Regulated by Ly6C(low) F4/80(+) Extratumoral Macrophages. *Gastroenterology*. Jul 2015;149(1):201-10.
139. Troiano G, Caponio VCA, Adipietro I, et al. Prognostic significance of CD68(+) and CD163(+) tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. *Oral Oncol*. Jun 2019;93:66-75.
140. López-Janeiro Á, Padilla-Ansala C, de Andrea CE, Hardisson D, Melero I. Prognostic value of macrophage polarization markers in epithelial neoplasms and melanoma. A systematic review and meta-analysis. *Mod Pathol*. Aug 2020;33(8):1458-1465.
141. Wang C, Lin Y, Zhu H, et al. The Prognostic and Clinical Value of Tumor-Associated Macrophages in Patients With Breast Cancer: A Systematic Review and Meta-Analysis. *Front Oncol*. 2022;12:905846.
142. Van Laethem JL, Borbath I, Prenen H, et al. Combining CD40 agonist mitazalimab with mFOLFIRINOX in previously untreated metastatic pancreatic ductal adenocarcinoma (OPTIMIZE-1): a single-arm, multicentre phase 1b/2 study. *Lancet Oncol*. Jul 2024;25(7):853-864.
143. O'Hara MH, O'Reilly EM, Varadhachary G, et al. CD40 agonistic monoclonal antibody APX005M (sotigalimab) and chemotherapy, with or without nivolumab, for the treatment of metastatic pancreatic adenocarcinoma: an open-label, multicentre, phase 1b study. *Lancet Oncol*. Jan 2021;22(1):118-131.
144. Padrón LJ, Maurer DM, O'Hara MH, et al. Sotigalimab and/or nivolumab with chemotherapy in first-line metastatic pancreatic cancer: clinical and immunologic analyses from the randomized phase 2 PRINCE trial. *Nat Med*. Jun 2022;28(6):1167-1177.
145. Van Laethem JL, Geboes K, Borbath I, et al. CD40 agonist mitazalimab with mFOLFIRINOX in untreated metastatic pancreatic cancer: Biomarkers associated with outcomes from OPTIMIZE-1. *Cell Rep Med*. Oct 21 2025;6(10):102407.
146. Beatty GL, Torigian DA, Chiorean EG, et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res*. Nov 15 2013;19(22):6286-95.

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147. Winograd R, Byrne KT, Evans RA, et al. Induction of T-cell Immunity Overcomes Complete Resistance to PD-1 and CTLA-4 Blockade and Improves Survival in Pancreatic Carcinoma. *Cancer Immunol Res*. Apr 2015;3(4):399-411.
148. Morrison AH, Diamond MS, Hay CA, Byrne KT, Vonderheide RH. Sufficiency of CD40 activation and immune checkpoint blockade for T cell priming and tumor immunity. *Proc Natl Acad Sci U S A*. Apr 7 2020;117(14):8022-8031.
149. Burrack AL, Rollins MR, Spartz EJ, et al. CD40 Agonist Overcomes T Cell Exhaustion Induced by Chronic Myeloid Cell IL-27 Production in a Pancreatic Cancer Preclinical Model. *J Immunol*. Mar 15 2021;206(6):1372-1384.
150. Sanborn R, Gabrail N, Carneiro B, et al. Abstract 596: Results from a phase 1 study of CDX-1140, a fully human anti-CD40 agonist monoclonal antibody (mAb), in combination with pembrolizumab. *Journal for ImmunoTherapy of Cancer*. 2022;10(Suppl 2)doi:10.1136/jitc-2022-SITC2022.0596
151. Sanborn RE, Gabrail NY, Bhardwaj N, et al. Abstract LB-194: First-in-human Phase I study of the CD40 agonist mAb CDX-1140 and in combination with CDX-301 (rhFLT3L) in patients with advanced cancers: Interim results. *Cancer Research*. 2019;79(13_Supplement):LB-194-LB-194. doi:10.1158/1538-7445.AM2019-LB-194
152. Hogg GD, Weinstein AG, Kingston NL, et al. Combined Flt3L and CD40 agonism restores dendritic cell-driven T cell immunity in pancreatic cancer. *Sci Immunol*. Aug 15 2025;10(110):eadp3978.
153. Fu S. Abstract e14501: Early safety and efficacy from a phase I open-label clinical study of LVGN7409 (CD40 agonist antibody) in patients with advanced or metastatic malignancies. presented at: ASCO Annual Meeting; 2022; Session Developmental Therapeutics—Immunotherapy. Accessed 4/28/2026.
154. Sanchez-Paulete AR, Labiano S, Rodriguez-Ruiz ME, et al. Deciphering CD137 (4-1BB) signaling in T-cell costimulation for translation into successful cancer immunotherapy. *Eur J Immunol*. Mar 2016;46(3):513-22.
155. Schmiechen ZC, Nanda HA, Burrack AL, et al. IL-15 Complex Enhances Agonistic Anti-CD40 + Anti-PDL1 by Correcting the T-bet to Tox Ratio in CD8+ T cells Infiltrating Pancreatic Ductal Adenocarcinoma. *Cancer Immunol Res*. Jun 4 2025;13(6):847-866.
156. Rojas LA, Sethna Z, Soares KC, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature*. Jun 2023;618(7963):144-150.
157. Pant S, Wainberg ZA, Weekes CD, et al. Lymph-node-targeted, mKRAS-specific amphiphile vaccine in pancreatic and colorectal cancer: the phase 1 AMPLIFY-201 trial. *Nat Med*. Feb 2024;30(2):531-542.
158. Sethna Z, Guasp P, Reiche C, et al. RNA neoantigen vaccines prime long-lived CD8(+) T cells in pancreatic cancer. *Nature*. Mar 2025;639(8056):1042-1051.
159. Vonderheide RH. The Immune Revolution: A Case for Priming, Not Checkpoint. *Cancer Cell*. Apr 9 2018;33(4):563-569.
160. Yamauchi T, Hoki T, Oba T, et al. CD40 and CD80/86 signaling in cDC1s mediate effective neoantigen vaccination and generation of antigen-specific CX3CR1(+) CD8(+) T cells. *Cancer Immunol Immunother*. Jan 2022;71(1):137-151.
161. Kucukcelebi S, van 't Land FR, van der Burg SH, et al. REACtiVe-2: phase I evaluation of dendritic cell vaccination and agonistic CD40 therapy following (m)FOLFIRINOX in metastatic pancreatic cancer. *Nat Commun*. Nov 28 2025;16(1):10609.
162. Zhao J, Wen X, Tian L, et al. Irreversible electroporation reverses resistance to immune checkpoint blockade in pancreatic cancer. *Nat Commun*. Feb 22 2019;10(1):899.
163. Sabel MS, Yamada M, Kawaguchi Y, Chen FA, Takita H, Bankert RB. CD40 expression on human lung cancer correlates with metastatic spread. *Cancer Immunol Immunother*. May 2000;49(2):101-8.
164. Ishikawa K, Miyamoto M, Yoshioka T, et al. Up-regulation of CD40 with juxtacrine activity in human nonsmall lung cancer cells correlates with poor prognosis. *Cancer*. Aug 1 2008;113(3):530-41.
165. Guo J, Xiao JJ, Zhang X, Fan KX. CD40 expression and its prognostic significance in human gastric carcinoma. *Med Oncol*. Mar 2015;32(3):63.
166. Werner JM, Kuhl S, Ulrich K, et al. Expression of CD40 Correlates Negatively with Overall and Progression-Free Survival of Low- and High-Grade Gliomas. *World Neurosurg*. Oct 2019;130:e17-e25.

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167. Grazia GA, Bastos DR, Villa LL. CD40/CD40L expression and its prognostic value in cervical cancer. *Braz J Med Biol Res*. 2023;56:e13047.
168. Uehara Y, Nishizaki D, Fujiwara Y, et al. CD40 transcriptomic expression patterns across malignancies: implications for clinical trials of CD40 agonists. *Cancer Immunol Immunother*. Nov 3 2025;74(12):359.
169. Digomann D, Heiduk M, Reiche C, et al. Serum immune checkpoint profiling identifies soluble CD40 as a biomarker for pancreatic cancer. *NPJ Precis Oncol*. Oct 14 2023;7(1):104.
170. Vonderheide RH, Flaherty KT, Khalil M, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J Clin Oncol*. Mar 1 2007;25(7):876-83.
171. Rüter J, Antonia SJ, Burris HA, Huhn RD, Vonderheide RH. Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. *Cancer Biol Ther*. Nov 15 2010;10(10):983-93.
172. Richman LP, Vonderheide RH. Role of crosslinking for agonistic CD40 monoclonal antibodies as immune therapy of cancer. *Cancer Immunol Res*. Jan 2014;2(1):19-26.
173. Eriksson E, Milenova I, Wenthe J, Moreno R, Alemany R, Loskog A. IL-6 Signaling Blockade during CD40-Mediated Immune Activation Favors Antitumor Factors by Reducing TGF-β, Collagen Type I, and PD-L1/PD-1. *J Immunol*. Feb 1 2019;202(3):787-798.
174. Plesca I, Benešová I, Beer C, et al. Clinical Significance of Tumor-Infiltrating Conventional and Plasmacytoid Dendritic Cells in Pancreatic Ductal Adenocarcinoma. *Cancers (Basel)*. Feb 26 2022;14(5)
175. Tanaka T, Masuda A, Inoue J, et al. Integrated analysis of tertiary lymphoid structures in relation to tumor-infiltrating lymphocytes and patient survival in pancreatic ductal adenocarcinoma. *J Gastroenterol*. Mar 2023;58(3):277-291.
176. Kinker GS, Vitiello GAF, Diniz AB, et al. Mature tertiary lymphoid structures are key niches of tumour-specific immune responses in pancreatic ductal adenocarcinomas. *Gut*. Oct 2023;72(10):1927-1941.
177. Yousuf S, Qiu M, Voith von Voithenberg L, et al. Spatially Resolved Multi-Omics Single-Cell Analyses Inform Mechanisms of Immune Dysfunction in Pancreatic Cancer. *Gastroenterology*. Oct 2023;165(4):891-908 e14.
178. Hwang WL, Jagadeesh KA, Guo JA, et al. Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. *Nat Genet*. Aug 2022;54(8):1178-1191.
179. Mi H, Sivagnanam S, Betts CB, et al. Quantitative Spatial Profiling of Immune Populations in Pancreatic Ductal Adenocarcinoma Reveals Tumor Microenvironment Heterogeneity and Prognostic Biomarkers. *Cancer Res*. Dec 2 2022;82(23):4359-4372.
180. Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. *Clin Cancer Res*. Mar 1 2013;19(5):1035-43.
181. Liu HC, Davila Gonzalez D, Viswanath DI, et al. Sustained Intratumoral Administration of Agonist CD40 Antibody Overcomes Immunosuppressive Tumor Microenvironment in Pancreatic Cancer. *Adv Sci (Weinh)*. Mar 2023;10(9):e2206873.
182. Chen S, Lin Y, Li D, et al. Development of a Tumor-Specific Multivalent CD40 Agonist Antibody FAPxCD40 for Cancer Therapy: Balancing Efficacy and Toxicity. *Mol Cancer Ther*. Jul 2 2025;24(7):1063-1074.
183. McIntyre CA, Grimont A, Park J, et al. Distinct clinical outcomes and biological features of specific KRAS mutants in human pancreatic cancer. *Cancer Cell*. Sep 9 2024;42(9):1614-1629 e5.
184. Chen Y, Shen X, Tang Y, et al. The diverse pancreatic tumor cell-intrinsic response to IFNγ is determined by epigenetic heterogeneity. *Cancer Lett*. May 28 2023;562:216153.
185. Osorio JC, Knorr DA, Weitzenfeld P, et al. Fc-optimized CD40 agonistic antibody elicits tertiary lymphoid structure formation and systemic antitumor immunity in metastatic cancer. *Cancer Cell*. Oct 13 2025;43(10):1902-1916 e9.
186. Wang LM, Yang C, Sun X, et al. SHR-2005, an FcγR-Dependent CD40 Agonistic Antibody with Potent Antitumor Activities and Favorable Safety Profile. *Mol Cancer Ther*. Nov 3 2025;24(11):1671-1684.
187. Ceglia V, Zurawski S, Montes M, et al. Anti-CD40 Antibodies Fused to CD40 Ligand Have Superagonist Properties. *J Immunol*. Oct 15 2021;207(8):2060-2076.

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188. Salomon R, Dahan R. Next Generation CD40 Agonistic Antibodies for Cancer Immunotherapy. *Front Immunol*. 2022;13:940674.
189. Sum E, Rapp M, Fröbel P, et al. Fibroblast Activation Protein α-Targeted CD40 Agonism Abrogates Systemic Toxicity and Enables Administration of High Doses to Induce Effective Antitumor Immunity. *Clin Cancer Res*. Jul 15 2021;27(14):4036-4053.
190. Althobaiti S, Parajuli P, Luong D, et al. Enhanced safety and efficacy profile of CD40 antibody upon encapsulation in pHe-triggered membrane-adhesive nanoliposomes. *Nanomedicine (Lond)*. Jan 2025;20(2):155-166.
191. Rech AJ, Dada H, Kotzin JJ, et al. Radiotherapy and CD40 Activation Separately Augment Immunity to Checkpoint Blockade in Cancer. *Cancer Res*. Aug 1 2018;78(15):4282-4291.
192. Maltez VI, Arora C, Gribbin KP, et al. Agonistic anti-CD40 antibody treatment converts resident regulatory T cells into activated type 1 effectors within the tumor microenvironment. *Immunity*. Apr 14 2026;59(4):1058-1074 e7.
193. Wartenberg M, Cibin S, Zlobec I, et al. Integrated Genomic and Immunophenotypic Classification of Pancreatic Cancer Reveals Three Distinct Subtypes with Prognostic/Predictive Significance. *Clin Cancer Res*. Sep 15 2018;24(18):4444-4454.
194. Guo JA, Hoffman HI, Weekes CD, Zheng L, Ting DT, Hwang WL. Refining the Molecular Framework for Pancreatic Cancer with Single-cell and Spatial Technologies. *Clin Cancer Res*. Jul 15 2021;27(14):3825-3833.
195. Steele NG, Carpenter ES, Kemp SB, et al. Multimodal Mapping of the Tumor and Peripheral Blood Immune Landscape in Human Pancreatic Cancer. *Nat Cancer*. Nov 2020;1(11):1097-1112.
196. Karamitopoulou E. Tumour microenvironment of pancreatic cancer: immune landscape is dictated by molecular and histopathological features. *Br J Cancer*. Jul 2019;121(1):5-14.
197. Kim H, Choi JH, Lim Y, et al. Artificial Intelligence-Powered Spatial Analysis of Immune Phenotypes in Resected Pancreatic Cancer. *JAMA Surg*. Aug 1 2025;160(8):884-892.
198. Cao L, Huang C, Cui Zhou D, et al. Proteogenomic characterization of pancreatic ductal adenocarcinoma. *Cell*. Sep 16 2021;184(19):5031-5052 e26.
199. Coveler AL, Smith DC, Phillips T, et al. Phase 1 dose-escalation study of SEA-CD40: a non-fucosylated CD40 agonist, in advanced solid tumors and lymphomas. *J Immunother Cancer*. Jun 2023;11(6)

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