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Manipulating regulatory T cells: is it the key to unlocking effective immunotherapy for pancreatic ductal adenocarcinoma?

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The five-year survival rates for pancreatic ductal adenocarcinoma (PDAC) have scarcely improved over the last half-century. It is inherently resistant to FDA-approved immunotherapies, which have transformed the outlook for patients with other advanced solid tumours. Accumulating evidence relates this resistance to its hallmark immunosuppressive milieu, which instils progressive dysfunction among tumour-infiltrating effector T cells. This milieu is established at the inception of neoplasia by immunosuppressive cellular populations, including regulatory T cells (T_{regs}), which accumulate in parallel with the progression to malignant PDAC. Thus, the therapeutic manipulation of T_{regs} has captured significant scientific and commercial attention, bolstered by the discovery that an abundance of tumour-infiltrating T_{regs} correlates with a poor prognosis in PDAC patients. Herein, we propose a mechanism for the resistance of PDAC to anti-PD-1 and CTLA-4 immunotherapies and re-assess the rationale for pursuing T_{reg}-targeted therapies in light of recent studies that profiled the immune landscape of patient-derived tumour samples. We evaluate strategies that are emerging to limit T_{reg}-mediated immunosuppression for the treatment of PDAC, and signpost early-stage trials that provide preliminary evidence of clinical activity. In this context, we find a compelling argument for investment in the ongoing development of T_{reg}-targeted immunotherapies for PDAC.

KEYWORDS

immunotherapy, regulatory T cells, pancreatic ductal adenocarcinoma, TIGIT, CCR8, Helios, adenosine

1 Introduction

Since 1863 – when Rudolf Virchow first observed leukocyte infiltrates decorating neoplastic tissues – research has uncovered a dynamic interplay between the immune system and pre-malignant cells, which governs their progressive transformation to invasive derivatives (1). In parallel, efforts to leverage the immune system to treat malignancy have a long history; in 1868, Wilhelm Busch reported tumour regression after intentionally infecting patients with *Streptococcus pyogenes* (2). Today, immunotherapy has revolutionised clinical oncology: immune checkpoint inhibitors (ICIs; specifically anti-PD-1, -CTLA-4, and -PD-L1 antibodies) provide unprecedented rates of durable anti-tumour responses in patients with several types of cancer (3). However, ICIs, including the combination of anti-CTLA-4 and anti-PD-L1 antibodies, have yielded limited responses in pancreatic ductal adenocarcinoma (PDAC); a malignancy of the exocrine pancreas that constitutes 95% of pancreatic cancer cases (4, 5). Accordingly, PDAC carries a bleak prognosis: globally, the 5-year survival rate at the time of diagnosis is 9% (6).

Substantial research has sought to identify immunological mechanisms that render PDAC resistant to ICIs. Concomitantly, these studies have unearthed therapeutic targets that could feasibly be exploited to induce anti-tumour immunity in PDAC; indeed, strategies to restrain immunosuppressive regulatory T cells (T_{regs}), myeloid cells, and cancer-associated fibroblasts are currently under development (7, 8). The manipulation of $CD4^+$ T_{regs} has gained considerable traction, stemming from the discovery that an abundance of intratumoral T_{regs} correlates with a poor prognosis in PDAC patients (9). Herein, we propose a mechanism for the intrinsic resistance of PDAC to ICIs; discuss the rationale for pursuing T_{reg}-targeted therapies in the context of PDAC; and evaluate emerging strategies to limit T_{reg}-mediated immunosuppression. Overall, we argue that T_{reg}-targeted immunotherapies offer a valuable opportunity to improve clinical outcomes in PDAC.

2 Why have ICIs proved ineffective in the context of PDAC?

Any effective immunotherapy must induce lasting anti-tumour immunity, typically mediated by $CD4^+$ and $CD8^+$ effector T (T_{eff}) cells and directed against tumour-associated antigens acquired during malignant progression (10, 11). Researchers have sought to identify immunological mechanisms that render PDAC resistant to ICIs. Initial efforts utilised autochthonous murine models of PDAC: *Kras*$^{LSL\text{-}G12D/+}$;*Pdx-1-Cre* (KC) and *Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{LSL\text{-}R172H/+}$;*Pdx-1-Cre* (KPC), which recapitulate features of the human disease (12, 13). More recent analyses have profiled the immune landscape of patient-derived tumour samples, facilitated by advances in single-cell multi-omic technologies (14–16).

It is well established that the baseline density of tumour-infiltrating T_{eff} cells is a critical determinant of therapeutic responses to ICIs (17, 18). Thus, the immunologically 'cold' phenotype that characterises PDAC has often been attributed to the physical exclusion of T_{eff} cells from the tumour microenvironment (TME) (19, 20). However, recent studies have challenged this paradigm, identifying heterogenous baseline infiltrates of $CD4^+$ and $CD8^+$ T_{eff} cells that correlate with prolonged overall survival in PDAC patients (14, 15, 21–26). There is also evidence for ongoing anti-tumour immunity; Freed-Pastor et al. identified a population of HLA-$DR^+Ki67^+CD57^-CD8^+$ T cells – indicative of an activated, proliferative phenotype – that are present in the majority of patients (27). Altogether, these studies suggest that inducing T_{eff} cell-mediated anti-tumour immunity in PDAC may not be as intractable as is widely considered (23).

In further support of this notion, a rare subset (~1.6.%) of PDAC patients with hypermutated mismatch repair deficient (dMMR) tumours exhibit marked therapeutic responses to anti-PD-1 antibodies (28). These tumours present a broad repertoire of neoantigens, which direct potent anti-tumour immune responses (29, 30). Indeed, in this patient cohort, sequencing of the TCR Vβ chain revealed that 94% of intratumoral T cell clonotypes were unique to tumours, implying the existence of a neoantigen-specific immune response (24). Overall, this highlights the importance of neoantigens as a substrate for T_{eff}-mediated anti-tumour immunity – indeed, on the basis of this principle, pembrolizumab and nivolumab (anti-PD-1) were granted FDA-approval in 2017 for the treatment of dMMR tumours, irrespective of their tissue of origin (31). In this context, it is notable that recent studies have challenged the claim that MMR-proficient PDAC harbours a limited repertoire of neoantigens. Freed-Pastor et al. investigated a cohort of 57 advanced PDAC patients and discovered that they all possessed neoepitopes with predicted ability to bind MHC class-I molecules (27). Accordingly, studies have consistently identified intratumoral neoantigen-reactive $CD8^+$ T cells in PDAC patients, indicating that these neoantigens are capable of directing anti-tumour immunity (27, 32, 33).

Nevertheless, it is evident that this population of intratumoral neoantigen-reactive $CD8^+$ T cells is not sufficient to drive therapeutic responses to FDA-approved ICIs in MMR-proficient PDAC. Indeed, multi-omic profiling of the PDAC immune landscape in resectable patients has revealed that 'dysfunctional' and 'senescent' phenotypes – both hypofunctional states, defined by the expression of multiple inhibitory receptors: TIGIT, LAG-3, TIM-3, and CD39 – dominate the intratumoral T_{eff} cell repertoire, leaving few activated T cells that are thus unable to control the tumour (15, 25). In addition, a more pronounced exhaustion signature has been observed in $CD8^+$ T cells from fine-needle biopsy samples of advanced, unresectable PDAC (14).

This progressive dysfunction of intratumoral T_{eff} cells can be attributed to the profoundly immunosuppressive TME. It is established by the progressive infiltration of immunosuppressive cells: T_{regs}, myeloid-derived suppressor cells, neutrophils, and tumour-associated macrophages (34). In the murine KC model, these populations dominate the immune landscape of pancreatic intraepithelial neoplasia (PanIN): precursor lesions that culminate in the development of PDAC (19). Other non-immune cellular populations also contribute to the immunosuppressive TME. For example, a subset of cancer-associated fibroblasts present antigenic

peptides in association with MHC class-II molecules; however, they lack expression of classical co-stimulatory molecules and thus command $CD4^+$ T cells to the T_{reg} lineage (35). In summary, neoantigen-specific T_{eff} responses are dampened by the gradual accumulation of immunosuppressive cells in the TME, which dictates the progression from PanIN to PDAC. Hence, the development of immunomodulatory therapies for PDAC must focus on surmounting the hallmark immunosuppressive TME (36). Importantly, the progressive nature of intratumoral T_{eff} cell dysfunction promises to confer a broad window during which such therapies might be effective.

3 What is the phenotype of T_{regs} in PDAC?

To date, strategies targeting myeloid-derived suppressor cells or cancer-associated fibroblasts for the treatment of PDAC have generally failed to demonstrate therapeutic promise in clinical trials (37–40). However, one promising strategy – which has gained substantial traction in the context of PDAC – is combatting T_{reg}-mediated immunosuppression. This originated from the discovery that an abundance of intratumoral T_{regs} correlates with a poorer prognosis in PDAC patients (9). Accordingly, the depletion of T_{regs} has been shown to delay tumour growth in orthotopically transplanted murine PDAC, albeit with conflicting results from other murine models (41, 42). However, recent single-cell analyses have uncovered extensive diversity among intratumoral T_{regs}; in this context, it is important to re-evaluate the rationale for the development of T_{reg}-targeted therapies.

3.1 Effector T_{regs} are highly immunosuppressive

Classically, $CD4^+$ T_{regs} have been defined according to expression of FOXP3, considered a lineage-specifying transcription factor (TF), or the interleukin (IL)-2 receptor α chain (CD25). In a seminal study, Hiraoka et al. discovered that the prevalence of $FOXP3^+$ T_{regs} increases during the progression from PanIN to advanced PDAC – at this latter stage, they constitute 35% (± 11%) of the total intratumoral $CD4^+$ population (9, 15). Further, it is estimated that 54% (± 19%) of intratumoral T_{regs} are effector T_{regs} (eT_{regs}; $CD45RA^-FOXP3^{hi}CD25^{hi}$) (15). These cells express high levels of TIGIT, CTLA-4, ICOS, CD39, and HLA-DR, which are indicative of functional activation and potent immunosuppressive capacity (14, 15). This activated state has been attributed to sustained TCR stimulation, provided by the plethora of self- and quasi-self-antigens present in the inflammatory TME (43). However, a stable eT_{reg} phenotype is also dependent on the expression of Helios, a member of the Ikaros TF family. Indeed, intratumoral $Helios^+$ T_{regs} exhibit significantly higher expression of FOXP3, compared to $Helios^-$ T_{regs} (44).

Intratumoral eT_{regs} potently suppress $CD8^+$ T cell-mediated immunity via the expression of co-inhibitory molecules e.g., CTLA-4, which prevents the functional maturation of dendritic cells (41); secretion of immunosuppressive cytokines e.g., IL-10, IL-35, and TGF-*β*; sequestration of IL-2, which hampers IL-2-dependent T cell activation; and the secretion of granzymes to lyse target $CD8^+$ cells (45). In support of their immunosuppressive capacity *in situ*, spatial analyses reveal that 90% of T_{regs} reside in close proximity to a $CD8^+$ T cell in the PDAC TME (15).

3.2 $FOXP3^+RORγt^+$ T_{regs} provide mitogenic signalling

$FOXP3^+$ T_{regs} exhibit extensive heterogeneity in PDAC. Strikingly, studies have discovered populations of $FOXP3^+$ T_{regs} that, in addition to IL-10, secrete high levels of pro-inflammatory cytokines. For example, Chellappa et al. identified T_{regs} that co-express FOXP3 and RORγt: a factor that specifies the type-17 T-helper cell lineage (T_H17) (46). These cells retained markers associated with $FOXP3^+$ T_{regs} e.g., CTLA-4, CD39, and ICOS, indicating an ability to robustly suppress anti-tumour immunity. However, through the simultaneous production of IL-17, these $FOXP3^+RORγt^+$ cells provide mitogenic signalling to transformed pancreatic epithelial cells, which upregulate the IL-17 receptor (47). Moreover, studies have identified populations of $FOXP3^-$ T_{reg}-like cells that share expression of molecules classically associated with immunosuppressive T_{reg} functions (e.g., IL-10, CCR8, TIGIT, ICOS, CTLA-4) (48, 49). Barilla et al. demonstrated that the gene expression profile of one such population, termed T_r1 cells (*CD49b*, *CD73*, and *AHR*), was associated with decreased overall survival in PDAC patients (49). Furthermore, Whiteside et al. suggest that intratumoral T_{eff} cells may adopt this $FOXP3^-$ T_{reg}-like phenotype following the ablation of $FOXP3^+$ cells (48).

This profound heterogeneity likely explains conflicting reports regarding the overall contribution of T_{regs} in the pathophysiology of PDAC. One notable study reported an increased prevalence of T_{regs} in tumours of long-term PDAC survivors (24). Moreover, depletion of T_{regs} prior to the development of PanIN in KC mice has been shown to accelerate malignant progression (42). Conceivably, the use of different experimental systems, including varied methods for detecting and defining intratumoral T_{regs}, might accentuate specific T_{reg}-associated functions and thereby explain these conflicting reports. Moreover, studies have suggested that, as part of normal immune homeostasis, intratumoral T_{regs} accompany $CD8^+$ T cell infiltrates (21, 42, 49), which may further obscure any relationship between the prevalence of intratumoral T_{regs} and a poor prognosis. Nevertheless, harnessing the therapeutic manipulation of T_{regs} will require a targeted approach, based on a detailed understanding of the heterogeneous functions ascribed to T_{regs}, and their spatiotemporal dynamics in the PDAC TME (Figure 1). In addition, such an approach will reduce the systemic side-effects associated with T_{reg}-targeted immunotherapies.

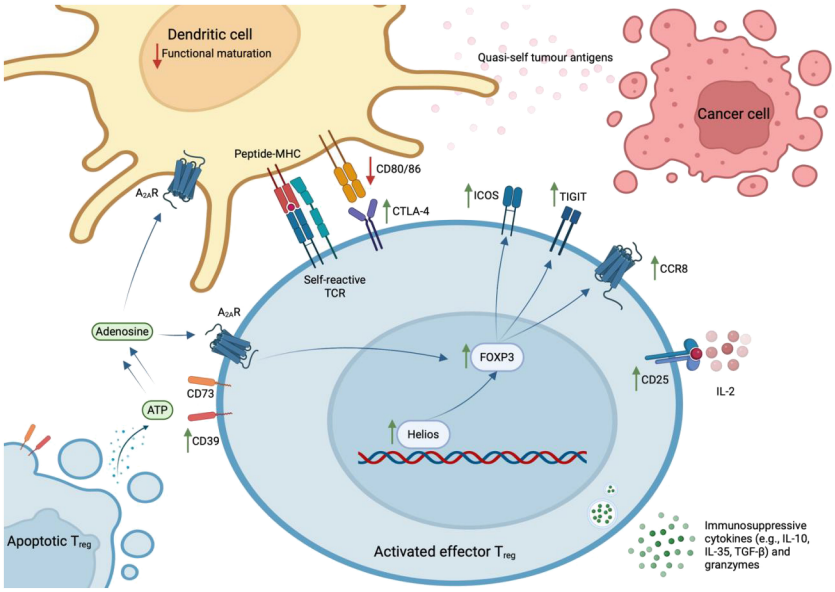

FIGURE 1
Phenotype of effector T_{reg} cells in human PDAC. Effector T_{regs} – characterised by the expression of FOXP3, CD25, TIGIT, CTLA-4, ICOS, CD39, and CCR8 – are activated by sustained TCR stimulation with abundant self- and quasi-self-antigens and stabilised by expression of the Helios transcription factor. These cells exhibit potent immunosuppressive capacity within the PDAC TME, where they exist in close proximity to $CD8^+$ T lymphocytes. Specifically, they express co-inhibitory molecules (e.g., CTLA-4, TIGIT, ICOS); convert ATP to immunosuppressive adenosine via ectoenzymes that remain catalytically active after cell-death (CD39 and CD73); secrete immunosuppressive cytokines (e.g., IL-10, IL-35, TGF-β) and granzymes that lyse $CD8^+$ T_{eff} cells; and sequester IL-2 that is required for T_{eff} cell activation.

3.3 Apoptotic T_{regs} are paradoxically immunosuppressive

This hypothesis is fortified by the discovery that apoptotic T_{regs}, defined by increased expression of Ki67 and cleaved caspase-3, exert immunosuppressive effects in the oxidative TME. They release large quantities of ATP, which is converted into adenosine via CD39 and CD73 – ectoenzymes that are expressed by T_{regs} and remain catalytically active after cell-death (50). Through the A_{2A} receptor, accumulating extracellular adenosine inhibits T_{eff} cell proliferation; induces immunosuppressive dendritic cells; and stabilises surviving T_{regs} (51). Thus, CD39 and CD73 expression correlate with a poor prognosis in patients with various solid tumours (52, 53). Importantly, this paracrine signalling pathway is likely to be operating in human PDAC, as intratumoral T_{regs} express high levels of CD39.

4 What are the strategies to manipulate T_{regs} for the treatment of PDAC?

The manipulation of T_{regs} has captured significant attention from both scientific and commercial communities as a novel approach to the treatment of PDAC. The earliest attempts depleted T_{regs} by targeting CD25 with antibodies, daclizumab, or the IL-2-diphtheria toxin fusion protein, ONTAK (54, 55). However, IL-2 signalling via CD25 promotes the survival of activated T_{eff} cells, conferring a limited therapeutic window to CD25-targeted interventions. Nevertheless, these efforts provided proof-of-concept for the therapeutic manipulation of T_{regs}. Today, numerous T_{reg}-targeted therapies are under development for the treatment of advanced solid tumours, including PDAC (Table 1).

4.1 Re-engineering next-generation ICIs

Allison and colleagues originally attributed the anti-tumour activity of anti-CTLA-4 monoclonal antibodies (mAbs) to the reinvigoration of dysfunctional T_{eff} cells (56). However, accumulating evidence suggests that anti-CTLA-4 mAbs can preferentially deplete CTLA-4^{hi} T_{regs} *in vivo* by antibody-dependent cellular cytotoxicity (ADCC) (57–60). Thus, in spite of the failure of prior clinical trials (4, 61), this novel mechanistic insight provides a rationale for the continued development of anti-CTLA-4 mAbs to treat PDAC. Clearly, however, this will necessitate re-engineering of existing anti-CTLA-4 mAbs; specifically, the fragment crystallisable (F_c) domain to enhance affinity for activatory $F_c\gamma$ receptors and decrease affinity for inhibitory receptors, thereby promoting ADCC. This approach can be optimised with consideration of the relative abundance and distribution of specific $F_c\gamma$Rs on local effector cells; indeed, the engineering of anti-CTLA-4 mAbs in this manner has been shown to increase therapeutic activity in tumour-bearing mice (59, 62). Therefore, it is important that studies have identified intratumoral populations of $F_c\gamma$RIIIA (CD16)-expressing natural killer and myeloid cells in human PDAC (14–16). Moreover, Agenus

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TABLE 1 T_{reg}-targeted immunotherapies in current development (as of 01/05/2024).

Drug	Sponsor	Properties	Status	Reference
CTLA-4				
Botensilimab (AGEN1181)	Agenus Inc.	Fc-engineered anti-CTLA-4 monoclonal antibody	Phase I/II, in combination with gemcitabine and nab-paclitaxel, in patients with metastatic PDAC	NCT05630183
ONC-392	OncoC4	Fc-engineered anti-CTLA-4 monoclonal antibody	Phase I/II, +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours, including PDAC	NCT04140526
XTX101	Xilio Therapeutics	Fc-engineered anti-CTLA-4 monoclonal antibody	Phase I/II, +/- atezolizumab (anti-PD-L1), in patients with advanced solid tumours	NCT04896697
TIGIT				
Tiragolumab (MTIG7192A)	Roche/Genentech	Anti-TIGIT monoclonal antibody	Phase I/II, in combination with atezolizumab (anti-PD-L1) and chemotherapy, in patients with metastatic PDAC	NCT03193190
Domvanalimab (AB154)	Arcus Biosciences/ Gilead Sciences	Fc-silent anti-TIGIT monoclonal antibody	Phase I/II trial, in combination with zimberelimab and APX005M (agonistic CD40), in patients with metastatic PDAC	NCT05419479
AB308	Arcus Biosciences/ Gilead Sciences	Fc-enabled anti-TIGIT monoclonal antibody	Phase Ib, in combination with zimberelimab (anti-PD-1), in patients with advanced solid tumours	NCT04772989
Vibostolimab (MK-7684)	Merck Sharp & Dohme	Anti-TIGIT monoclonal antibody	Phase I, +/- pembrolizumab (anti-PD-1) +/- chemotherapy, in patients with advanced solid tumours	NCT02964013
Belrestotug (EOS-448)	GlaxoSmithKline/ iTeos Therapeutics	Anti-TIGIT monoclonal antibody	Phase I/II, +/- pembrolizumab or dostarlimab (anti-PD-1) +/- inupadenant (selective A2aR antagonist) +/- chemotherapy, in patients with advanced solid tumours	NCT05060432
Ociperlimab (BGB-A1217)	BeiGene	Anti-TIGIT monoclonal antibody	Phase I, +/- tislelizumab (anti-PD-1) +/- chemotherapy, in patients with advanced solid tumours	NCT04047862
PM1021	Biotheus	Anti-TIGIT monoclonal antibody	Phase I, +/- PM8001 (anti-PDL1-TGFb), in patients with advanced solid tumours	NCT05537051
Etigilimab (MPH313)	Mereo BioPharma	Anti-TIGIT monoclonal antibody	Phase I/II, in combination with nivolumab (anti-PD-1), in patients with advanced solid tumours	NCT04761198

(Continued)

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TABLE 1 Continued

Drug	Sponsor	Properties	Status	Reference
TIGIT				
BAT6005	Bio-Thera Solutions	Anti-TIGIT monoclonal antibody	Phase I in patients with advanced or metastatic solid tumours	NCT05116709
HB0030	Shanghai Huaota Pharmaceuticals	Anti-TIGIT monoclonal antibody	Phase I in patients with advanced solid tumours	NCT05706207
JS006	Shanghai Junshi Biosciences	Anti-TIGIT monoclonal antibody	Phase I, +/- toripalimab (anti-PD-1), in patients with advanced solid tumours	NCT05061628
AK127	Akeso	Anti-TIGIT monoclonal antibody	Phase I/II, in combination with AK104 (anti-CTLA4-PD1 bispecific), in patients with advanced or metastatic solid tumours	NCT05021120
COM902	Compugen	Anti-TIGIT monoclonal antibody	Phase I, +/- COM701 (anti-PVRIG*), in patients with advanced solid tumours	NCT04354246
CHS-006	Coherus BioSciences	Anti-TIGIT monoclonal antibody	Phase I, in combination with toripalimab (anti-PD-1), in patients with advanced solid tumours	NCT05757492
BMS-986442	Bristol Myers Squibb/ Agenus Inc.	Anti-TIGIT bispecific antibody (other target is undisclosed)	Phase I/II, in combination with nivolumab (anti-PD-1) +/- chemotherapy, in patients with advanced solid tumours	NCT05543629
HB0036	Shanghai Huaota Pharmaceuticals	Anti-TIGIT-PDL1 bispecific antibody	Phase I/II in patients with advanced solid tumours	NCT05417321
PM1022	Biotheus	Anti-TIGIT-PDL1 bispecific antibody	Phase I/II in patients with advanced solid tumours	NCT05867771
PM1009	Biotheus	Anti-TIGIT-PVRIG bispecific antibody	Phase I in patients with advanced solid tumours	NCT05607563
HLX301	Shanghai Henlius Biotech	Anti-TIGIT-PDL1 bispecific antibody	Phase I/II in patients with advanced or metastatic solid tumours	NCT05102214
HLX53	Shanghai Henlius Biotech	Anti-TIGIT Fc fusion protein	Phase I in patients with advanced solid tumours	NCT05394168
ICOS				
Alomfilimab (KY1044)	Kymab/Sanofi	Agonistic ICOS monoclonal antibody	Phase I/II, +/- atezolizumab (anti-PD-L1), in patients with advanced solid tumours including PDAC	NCT03829501
Vopratelimab (JTX-2011)	Jounce Therapeutics	Agonistic ICOS monoclonal antibody	Phase II, in combination with pimivalimab (anti-PD-1), in patients with non-small cell lung cancer	NCT04549025
Helios				
DKY709	Novartis Pharmaceuticals	Selective Helios degrader	Phase I, +/- PDR001 (anti-PD-1), in patients with advanced solid tumours	NCT03891953
PLX-4545	Plexium	Selective Helios degrader	Pre-clinical development	https://www.plexium.com/therapeutic-areas-plexium-e3-ligase-drugs/

(Continued)

TABLE 1 Continued

Drug	Sponsor	Properties	Status	Reference
Helios				
Helios CELMoD	Bristol Myers Squibb	Selective Helios degrader	Pre-clinical development	https://www.bms.com/researchers-and-partners/in-the-pipeline.html
CD25				
Vopikitug (RG6292)	Roche/Genentech	Anti-CD25 monoclonal antibody	Phase I, +/- atezolizumab (anti-PD-L1), in patients with advanced solid tumours	NCT04158583
AU-007	Aulos Bioscience Inc.	Anti-IL-2 monoclonal antibody	Phase I/II, +/- aldesleukin (recombinant IL-2), in patients with locally advanced or metastatic solid tumours	NCT05267626
CCR8				
BMS-986340	Bristol Myers Squibb	Non-fucosylated anti-CCR8 monoclonal antibody	Phase I/II, +/- nivolumab (anti-PD-1) +/- docetaxel, in patients with advanced solid tumours including PDAC	NCT04895709
CHS-114	Coherus BioSciences	Afucosylated anti-CCR8 monoclonal antibody	Phase I/II in patients with advanced solid tumours	NCT05635643
BAY3375968	Bayer	Afucosylated anti-CCR8 monoclonal antibody	Phase I, +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours	NCT05537740
GS-1811	Gilead Sciences	Afucosylated anti-CCR8 monoclonal antibody	Phase I, +/- zemberelimab (anti-PD-1), in patients with advanced solid tumours	NCT05007782
LM-108	LaNova Medicines	Fc-optimised anti-CCR8 monoclonal antibody	Phase I/II, +/- toripalimab (anti-PD-1), in patients with advanced solid tumours	NCT05518045
AMG-355	Amgen	Anti-CCR8 monoclonal antibody	Phase I, +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours	NCT06131398
S-531011	Shionogi	Anti-CCR8 monoclonal antibody	Phase I/II, +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours	NCT05101070
BGB-3055	BeiGene	Anti-CCR8 monoclonal antibody	Phase I, +/- tislelizumab (anti-PD-1), in patients with advanced or metastatic solid tumours	NCT05935098
Adenosinergic Pathway				
TTX-030	Trishula Therapeutics/AbbVie	Anti-CD39 monoclonal antibody	Phase II, + chemotherapy +/- budigalimab (anti-PD-1), in patients with metastatic PDAC	NCT06119217
ES002023	Elpiscience Biopharma	Anti-CD39 monoclonal antibody	Phase I in patients with advanced solid tumours, including PDAC	NCT05075564

(Continued)

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TABLE 1 Continued

Drug	Sponsor	Properties	Status	Reference
Adenosinergic Pathway				
PUR001	Purinomia Biotech	Anti-CD39 monoclonal antibody	Phase I in patients with advanced solid tumours	NCT05234853
JS019	Shanghai Junshi Biosciences	Anti-CD39 monoclonal antibody	Phase I in patients with advanced solid tumours	NCT05508373
AB598	Arcus Biosciences	Anti-CD39 monoclonal antibody	Phase I, +/- zimberelimab (anti-PD-1) +/- chemotherapy, in patients with advanced solid tumours	NCT05891171
ES014	Elpiscience Biopharma	Anti-CD39-TGFb bispecific antibody	Phase I in patients with advanced or metastatic solid tumours	NCT05717348
Oleclumab (MEDI9447)	AstraZeneca	Anti-CD73 monoclonal antibody	Phase II, in combination with chemotherapy and durvalumab (anti-PD-L1), in patients with resectable/ borderline resectable PDAC	NCT04940286
Mupadolimab (CPI-006)	Corvus Pharmaceuticals	Anti-CD73 monoclonal antibody	Phase Ib, +/- ciforadenant (selective A2aR antagonist) +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours including PDAC	NCT03454451
PT199	Phanes Therapeutics	Anti-CD73 monoclonal antibody	Phase I, +/- anti-PD-1 immunotherapy, in patients with advanced solid tumours including PDAC	NCT05431270
IPH5301	Innate Pharma	Anti-CD73 monoclonal antibody	Phase I, +/- trastuzumab (anti-HER2^{T}) and paclitaxel, in patients with advanced solid tumours including metastatic PDAC	NCT05143970
HB0045	Shanghai Huaota Pharmaceuticals	Anti-CD73 monoclonal antibody	Phase I/II in patients with advanced solid tumours including PDAC	NCT06056323
INCA00186	Incyte Corporation	Anti-CD73 monoclonal antibody	Phase I, +/- INCB106385 (dual A2aR/A2bR antagonist) +/- retifanlimab (anti-PD-1), in patients with advanced solid tumours	NCT04989387
SYM024	Symphogen	Anti-CD73 monoclonal antibody	Phase I, +/- Sym021 (anti-PD-1), in patients with advanced solid tumours	NCT04672434
Uliledlimab (TJ004309)	I-Mab	Anti-CD73 monoclonal antibody	Phase I, in combination with toripalimab (anti-PD-1), in patients with advanced solid tumours	NCT04322006
JAB-BX102	Jacobio Pharmaceuticals	Anti-CD73 monoclonal antibody	Phase I, +/- pembrolizumab (anti-PD-1), in patients with advanced solid tumours	NCT05174585

(Continued)

TABLE 1 Continued

Drug	Sponsor	Properties	Status	Reference
Adenosinergic Pathway				
Drebuxelimab (AK119)	Akeso	Anti-CD73 monoclonal antibody	Phase I, in combination with AK104 (anti-CTLA4-PD1 bispecific) or AK112 (anti-VEGF-PD1 bispecific[‡]), in patients with advanced solid tumours	NCT05559541, NCT05689853
PM1015	Biotheus	Anti-CD73 monoclonal antibody	Phase I in patients with advanced solid tumours	NCT05950815
Quemliclustat (AB680)	Arcus Biosciences/ Gilead Sciences	Small-molecule, selective CD73 antagonist	Phase I, in combination with nab-paclitaxel and gemcitabine +/- zimberelimab (anti-PD-1), in patients with advanced PDAC	NCT04104672
ATG-037	Antengene Therapeutics	Small-molecule, selective CD73 antagonist	Phase I, +/- pembrolizumab (anti-PD-1), in patients with locally advanced or metastatic solid tumours	NCT05205109
Dalutrafusp alfa (AGEN1423)	Agenus Inc.	Anti-CD73-TGFb bispecific antibody	Phase II, in combination with balstilimab (anti-PD-1) +/- chemotherapy, in patients with advanced PDAC	NCT05632328
Ciforadenant (CPI-444)	Corvus Pharmaceuticals/Vernalis	Small-molecule, selective A2aR antagonist	Phase Ib, in combination with mupadolimab (anti-CD73), in patients with advanced solid tumours including PDAC	NCT03454451
Inupadenant (EOS-850)	iTeos Therapeutics	Small-molecule, selective A2aR antagonist	Phase I in patients with advanced solid tumours	NCT05060432
TT-10 (PORT-6)	Portage Biotech	Small-molecule, selective A2aR antagonist	Phase I in patients with advanced solid tumours	NCT04969315
ILB-2109	Innolake Biopharm	Small-molecule, selective A2aR antagonist	Phase I in patients with locally advanced or metastatic solid tumours	NCT05278546
Etrumadenant (AB928)	Arcus Biosciences	Small-molecule, dual A2aR/ A2bR antagonist	Phase II, in combination with chemotherapy and atezolizumab (anti-PD-L1), in patients with metastatic PDAC	NCT03193190
TT-4 (PORT-7)	Portage Biotech	Small-molecule, selective A2bR antagonist	Phase I/II in patients with advanced solid tumours including PDAC	NCT04976660

* poliovirus receptor-related immunoglobulin domain-containing; † human epidermal growth factor receptor 2; ‡ vascular endothelial growth factor.
All information was obtained from the NIH clinical trials database (https://clinicaltrials.gov) or from the publicised development pipelines of pharmaceutical companies. The rows highlighted blue denote drugs that are under evaluation in clinical trials that include PDAC patients.

recently initiated a phase I/II trial to investigate botensilimab – an F_c-engineered anti-CTLA-4 mAb with enhanced affinity for $F_c\gamma$RIIIA – in metastatic PDAC patients (NCT05630183).

Further testament to the widespread interest in strategies to selectively deplete intratumoral T_{regs}, there is renewed attention on the development of anti-CD25 mAbs. For example, Solomon et al. developed an anti-CD25 mAb (RG6292) that selectively depletes $CD25^{hi}$ T_{regs}, whilst preserving CD25-STAT5 signalling required for T_{eff} cell-mediated anti-tumour immunity (63). Indeed, a phase I trial of RG6292, conducted in patients with advanced/metastatic solid tumours, indicated a manageable safety profile and preliminary anti-tumour activity (64). However, multi-omic analysis of patient-derived tumour samples obtained during treatment with RG6292 is required to confirm this proposed mechanism of action *in vivo*.

4.2 Exploiting novel immune checkpoints

Since the discovery of CTLA-4 and PD-1, studies have identified a plethora of immune checkpoints – both inhibitory (e.g., TIGIT, LAG-3, TIM-3) and co-stimulatory (e.g., ICOS, OX40, GITR, 4–1BB) – that might be exploited therapeutically to augment anti-tumour immunity. In PDAC, TIGIT and ICOS are expressed at high levels on intratumoral eT_{regs} (14, 15). TIGIT is also expressed, albeit at lower levels, by dysfunctional T_{eff} cells, whereas ICOS is induced upon the activation of intratumoral T_{eff} cells (14, 15, 27). Therefore, anti-TIGIT and agonistic ICOS mAbs might have a dual mechanism of action: the re-invigoration of dysfunctional T_{eff} cells and selective depletion of activated T_{regs} (65). However, achieving the optimal balance between these mechanisms will require F_c engineering to effectively engage specific F_c receptors (66).

Tiragolumab (IgG1κ anti-TIGIT) has demonstrated tolerability and preliminary anti-tumour activity in patients with advanced solid tumours (67, 68). Consequently, two early-stage trials are investigating anti-TIGIT mAbs, incorporated into combinatorial regimens, for the treatment of metastatic PDAC (NCT03193190, NCT05419479). By contrast, a phase I/II trial, investigating vopratelimab (IgG1κ agonistic ICOS) for the treatment of advanced solid tumours, including three PDAC patients, reported limited efficacy (69). However, on-treatment emergence of $ICOS^{hi}$ $CD4^+$ T_{eff} cells was associated with therapeutic responses, suggesting that vopratelimab might indeed re-invigorate dysfunctional T_{eff} cells in patients through ICOS activation. More generally, this illustrates that multi-omic analyses of on-treatment patient-derived samples during clinical trials may further advance our understanding of the PDAC immune landscape.

4.3 De-stabilising activated T_{regs}

The development of strategies for selectively drugging T_{regs} has been the subject of considerable research. One potential target is Helios; in PDAC patients, $Helios^+$ T_{regs} are significantly enriched in the TME (70). Moreover, T_{reg}-intrinsic deletion of Helios has been shown to enhance anti-tumour immunity in tumour-bearing mice (71). Interestingly, Helios-deficient T_{regs} acquire a T_{eff} phenotype including the production of pro-inflammatory cytokines (e.g., IFN-γ), which is attributed to downregulation of FOXP3 and de-repression of T_H1/T_H2 lineage determinants (43). In the absence of the stabilising influence of Helios, it appears that the inflammatory TME promotes the trans-differentiation of T_{regs} into activated T_{eff} cells. Intriguingly, this novel T_{eff} population is equipped with an inherently self-reactive TCR repertoire, which might be expected to direct a potent immune response against 'quasi-self' tumour antigens.

Transcription factors are traditionally considered difficult to drug. However, several recent studies have described small-molecules that selectively enhance the proteasomal degradation of Helios (72, 73). Future *in vivo* studies must determine whether these small-molecules can selectively destabilise activated intratumoral eT_{regs}; one clinical trial is currently evaluating this approach in advanced solid tumours (NCT03891953).

4.4 Targeting chemokine receptors

The origin of intratumoral $FOXP3^+$ T_{regs} is unclear – they may differentiate locally from T_{eff} cells or be recruited from the circulation. For the latter, targeting chemokine signalling axes (e.g., CCL2-CCR4; CCL5-CCR5) that can recruit T_{regs} into the PDAC TME is of interest. However, this strategy has proved disappointing thus far; clinical trials investigating mogamulizumab (IgG1 anti-CCR4) reported off-target depletion of T_H2/T_H17 cells, reflecting heterogeneous expression of CCR4 (74, 75).

It is notable, therefore, that intratumoral eT_{regs} uniquely express CCR8 (76). However, functional blockade of CCR8 does not affect T_{reg} recruitment; they acquire CCR8 expression in the TME, perhaps suggesting that this axis mediates retention of intratumoral T_{regs} (77). Nevertheless, CCR8 constitutes a target for the selective depletion of intratumoral eT_{regs} in PDAC. Pre-clinical studies have demonstrated that anti-CCR8 mAbs profoundly suppress tumour growth in tumour-bearing mice (76, 78). Further, this response coincided with the expansion of intratumoral $CD4^+$ T_{eff} cells and the preservation of systemic T_{reg} populations, which may mitigate the risk of autoimmune-related adverse events. Currently, eight early-stage trials are investigating anti-CCR8 mAbs for the treatment of advanced solid tumours (NCT04895709, NCT06131398, NCT05635643, NCT05537740, NCT05007782, NCT05518045, NCT05101070, NCT05935098).

4.5 Combatting immunosuppressive adenosine

Apoptotic T_{regs} convert ATP to adenosine, an immunosuppressive metabolite, via ectoenzymes that remain catalytically active after cell-death. This raises the paradoxical possibility that the therapeutic depletion of T_{regs} might not limit T_{reg}-cell-mediated immunosuppression. This discovery provided impetus to the development of immunotherapies that target the adenosinergic pathway: CD39, CD73, and the A_{2A}/A_{2B} receptors. It is hoped that these therapies will synergise with T_{reg}-targeted approaches, or other immunotherapeutic modalities, to induce potent anti-tumour immunity. To date, however, attempts to target this pathway with anti-CD73 mAbs have demonstrated no clinical benefit for PDAC patients; a phase-II trial investigating the combination of anti-CD73, anti-PD-L1, and chemotherapy revealed comparable efficacy to chemotherapy alone (79).

5 Conclusions and future perspectives

The manipulation of intratumoral T_{regs} may prove a valuable addition to our currently limited armamentarium for the treatment of PDAC. This therapeutic strategy has the potential to re-invigorate anti-tumour immunity by reprogramming the immunosuppressive milieu that is first established in pre-malignant lesions. This notion is supported by promising early-stage clinical trials of T_{reg}-targeted immunotherapies (68, 80). Moreover, data from trials investigating anti-CCR8 mAbs and

selective Helios degraders, strategies to selectively target intratumoral effector T_{regs}, are eagerly awaited.

There are several outstanding questions, however, which threaten to hinder the effective therapeutic manipulation of intratumoral T_{regs}:

1. Given that intratumoral T_{regs} are present from early carcinogenesis to the development of metastatic disease, are T_{reg}-targeted therapies effective in cohorts of patients from the full spectrum of the natural history of PDAC?
2. With novel T_{reg}-targeted interventions, is there on-treatment emergence of immunosuppressive FOXP3$^-$ T_{reg}-like cells (e.g., T_r1 cells) or other complementary immunosuppressive mechanisms?
3. How can we prevent immune-related adverse events, which so often necessitate treatment discontinuation, when targeting T_{regs} for the treatment of PDAC?
4. To what extent do T_{reg}-targeted therapies synergise with anti-cancer agents from our existing repertoire, including immunotherapies and conventional chemotherapies?

Importantly, with preliminary clinical evidence for the efficacy of T_{reg}-targeted therapies, there is a compelling argument for the allocation of resources to resolve these outstanding questions.

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Publisher's note

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