Mini-review

Overview of pre-clinical and clinical studies targeting angiogenesis in pancreatic ductal adenocarcinoma

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ABSTRACT

The importance of angiogenesis in pancreatic ductal adenocarcinoma (PDAC) and its therapeutic potential have been explored in both pre-clinical and clinical studies. Human PDACs overexpress a number of angiogenic factors and their cognate high-affinity receptors, and anti-angiogenic agents reduce tumor volume, metastasis, and microvessel density (MVD), and improve survival in subcutaneous and orthotopic pre-clinical models. Nonetheless, clinical trials using anti-angiogenic therapy have been overwhelmingly unsuccessful. This review will focus on these pre-clinical and clinical studies, the potential reasons for failure in the clinical setting, and ways these shortcomings could be addressed in future investigations of angiogenic mechanisms in PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), which comprises >85% of pancreatic cancers, is the 4th leading cause of cancer death in the United States with a 1- and 5-year relative survival of 28% and 7%, respectively [1–3]. These statistics are largely due to advanced stage at clinical presentation, the high frequency of major driver mutations, marked resistance to chemotherapy and radiation, and extensive desmoplasia that impedes drug delivery [4–8]. Because advances in screening, prevention, and treatment are limited compared to other cancers, PDAC is now projected to surpass breast, prostate, and colorectal cancers to become the second leading cause of cancer death by 2030 [9].

At presentation, only 15–20% of patients are eligible for surgical resection, the only chance for cure [1–3]. Even then, outcomes are poor, with a 5 year survival between 20 and 25% post-resection, since most of these patients develop disease recurrence [10]. Therefore, chemotherapy is recommended as adjuvant treatment for those undergoing surgical resection and is the mainstay of treatment for patients with locally advanced or metastatic disease [2]. The current standard of care for patients with metastatic disease includes gemcitabine plus nab-paclitaxel or fluorouracil plus leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) [2,11].

Angiogenesis

Blood vessel growth throughout adult life is primarily achieved via angiogenesis [12–18]. However, the adult vasculature is mostly
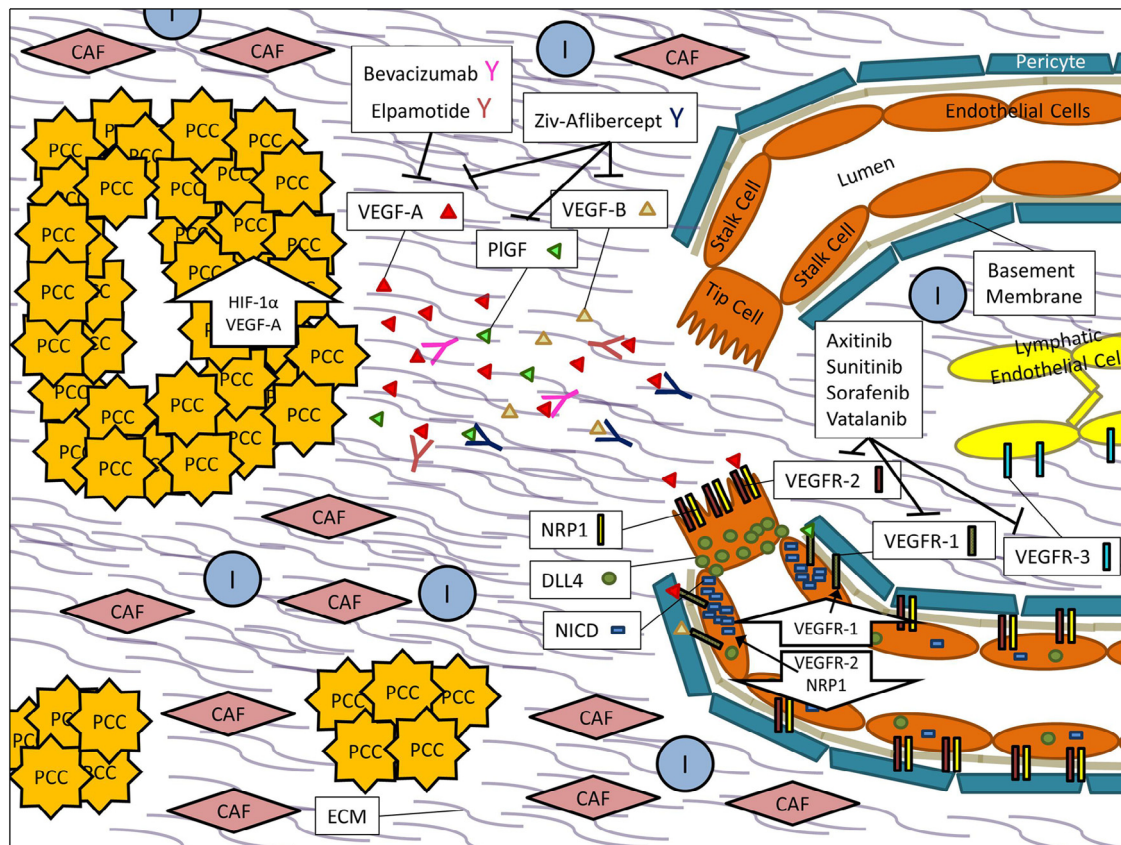
Fig. 1. PDAC angiogenesis. In PDAC, pancreatic cancer cells (PCCs) proliferate within a desmoplastic stroma that consists of both cellular components such as cancer associated fibroblasts (CAFs), immune cells (Is), and endothelial cells (ECs) as well as extracellular matrix (ECM) components like soluble growth factors, cytokines, collagens, fibronectin, laminin, glycoproteins, and proteoglycans. Up-regulation of hypoxia inducible factor 1, alpha subunit (gene: HIF1A) (HIF-1α) and the pro-angiogenic molecule VEGF-A within PCCs results in secretion of VEGF-A molecules into the tumor microenvironment. When VEGF-A signals through VEGFR-2 and its NRP1 co-receptor on endothelial cells, downstream signaling results in increased expression of DLL4. DLL4 will bind to Notch receptors on neighboring cells, subsequently releasing NICD, which then down-regulates VEGFR-2 and NRP1 expression and up-regulates expression of the VEGFR-1 decoy receptor. This favors migration of a tip cell toward the VEGF-A gradient while the neighboring stalk cells become de-sensitized to the signal. In the quiescent vasculature, DLL4 and Notch signaling are balanced. Small molecule inhibitors of angiogenesis, such as Axitinib, Sunitinib, Sorafenib, and Vatalanib primarily act on the vascular endothelial growth factor receptor complexes (VEGFR-1, VEGFR-2, and Vascular endothelial growth factor receptor 3 (gene: FLT4) (VEGFR-3)), while recombinant protein inhibitors of angiogenesis like Bevacizumab, Elpamotide, and Ziv-Aflibercept act on vascular endothelial growth factor ligands like VEGF-A, vascular endothelial growth factor B (gene: VEGFB) (VEGF-B), and/or placenta growth factor (gene: PGF) (PlGF).

quiescent as only 0.01% of the endothelium undergoes cell division at any time [12,13,15,17,18]. Examples of physiological angiogenesis in the adult include wound healing, tissues undergoing growth, exercise induced angiogenesis in heart and skeletal muscle, the hair cycle, skeletal growth, and female reproductive processes. Pathological examples include intraocular neovascular disorders, infantile hemangiomas, immunogenic rheumatoid arthritis, psoriasis, and tumorigenesis [12,13,16–20].

Through the use of models like the mouse retina, which becomes vascularized postnatally, we now understand many of the key players and processes involved in physiological angiogenesis [21]. In general, activation of endothelial cells by pro-angiogenic molecules leads to the detachment of pericytes from the endothelium and remodeling of the basement membrane and cell-to-cell junctions (Fig. 1) [22]. The best known pro-angiogenic molecule is vascular endothelial growth factor A (gene: VEGFA) (VEGF-A). VEGF-A binds to vascular endothelial growth factor receptor 2 (gene: KDR) (VEGFR-2) on endothelial cells, and its signaling is enhanced by the neuropilin-1 (NRP1) co-receptor, which facilitates complex internalization (Fig. 1) [22]. Downstream signaling results in increased expression of the Notch ligand delta-like protein 4 (DLL4), which binds to Notch receptors on neighboring endothelial cells (Fig. 1) [22]. This releases the notch intracellular domain (NICD) in these cells, which down-regulates VEGFR-2 and NRP1, and up-regulates vascular endothelial growth factor receptor 1 (gene: FLT1) (VEGFR-1), a decoy receptor for VEGF-A (Fig. 1) [22].

The goal of this process is to isolate one cell that will migrate toward the pro-angiogenic gradient (called the tip cell) while de-sensitizing neighboring cells to the same signal. It is believed that DLL4 and Notch signaling are balanced in the quiescent vasculature, and that tip cells will offset the balance in response to pro-angiogenic signals [14]. The cells adjacent to the tip cell are called stalk cells, and they proliferate behind the tip cell to elongate the sprout and form a lumen (Fig. 1) [22]. Once two tip cells on different sprouts meet, they will anastomose to form a perfused branch (Fig. 1) [22]. Basement membrane then forms, and pericytes are recruited to cover the vessel (Fig. 1) [22]. The process is dynamic in that endothelial cells will compete for the tip position with different cells displaying the phenotype over time.

Tumor angiogenesis

Whereas physiological angiogenesis is tightly controlled and comes to a resolution, pathological angiogenesis is abnormal and does not resolve [13,16,17,20,21]. Because cells need nutrients and oxygen from nearby capillaries to function and survive, early tumor growth is often restricted to a volume of only a few cubic millimeters until it is able to switch to an angiogenic phenotype [13,16,17,19,20,23,24]. Activation of angiogenesis occurs when pro-angiogenic molecules predominate over anti-angiogenic molecules, whereas inactivation occurs when the anti-angiogenic molecules dominate [12,13,25].

In tumorigenesis, the observed activation from a quiescent state is often described as an "angiogenic switch" [12,13,25].

The vessels formed during tumor angiogenesis are tortuous or disorganized, immature, and convoluted with excessive vessel branching lacking pericyte coverage rendering them fragile and leaky with bleeding and exudation of plasma proteins [15–18,21,22,24,26]. The distribution of new vessels in the tumor is also heterogeneous with some areas demonstrating intense neovascularization [15,19,20,22,26]. The vessels are often functionally defective with low blood flow and reduced oxygen delivery due to high interstitial pressure [15,18,22,26]. The resulting hypoxic environment exacerbates the pathological condition by further up-regulating pro-angiogenic molecules [15,22,26]. While one might assume that neovascularization would improve delivery of chemotherapeutic agents to the tumor, the poor perfusion and compression of the vascular supply actually impedes drug delivery [15,16,18,20,22]. Therefore, in addition to inhibiting angiogenesis and causing vessel regression, anti-angiogenic agents can enhance the effects of simultaneously administered chemotherapeutic drugs by normalizing the remaining vasculature [15,16,18,20–22,26].

PDAC is hypovascular

Though the previously discussed concepts are generalities common to many cancers, we now specifically consider concepts relevant to PDAC. Using the $Kras^{LSL-G12D/+}$, $Trp53^{LSL-R172H/+}$, Pdx-1-Cre (KPC) PDAC mouse model, which has oncogenic Kirsten rat sarcoma viral oncogene homolog (Kras) and mutated transformation related protein 53 (Trp53) in the pancreas due to Cre-mediated recombination, Olive et al. showed that KPC tumors are poorly vascularized, poorly perfused, and have impaired drug delivery when compared to KPC transplant models or normal mouse pancreas [27]. Likewise, using both $Kras^{LSL-G12D/+}$, Pdx-1-Cre (KC) mice, which have oncogenic Kras in the pancreas due to Cre-mediated recombination, and KPC mice, Provenzano et al. reported that in addition to having reduced vascularity, KC and KPC tumors have a paucity of large diameter (>10 um) vessels when compared to normal mouse pancreas [28]. This is likely due to vascular collapse caused by the presence of very high interstitial fluid pressures in these tumors, in the range of 75–130 mmHg, compared to 8–13 mmHg in normal mouse pancreas [28]. This observation also offers an explanation for the poor perfusion and drug delivery observed by Olive et al. [27]. Human PDAC samples were also shown to be poorly vascularized compared to normal human pancreas or adjacent normal human pancreas, and to have fewer large diameter vessels compared to adjacent normal human pancreas [27,28].

Because PDAC is inherently hypovascular, it might be assumed that this cancer either does not demonstrate significant angiogenesis or is not likely to benefit from anti-angiogenic agents. However, both concepts have been disproven in other cancers [29]. All tumor types need sufficient levels of nutrients and oxygen and are growth limited unless they are able to induce angiogenesis. This is also true of hypoxic tumors, which likely have increased requirements to drain away toxic by-products released by cancer cells. Instead of measuring angiogenesis, microvessel density (MVD) rather reflects the metabolic burden of the supported tumor cells [29]. In fact, because the oxygen consumption rate is often lower in tumors compared to the corresponding normal tissue, it is not uncommon for tumors to have lower MVDs as we see in PDAC [29]. This is also the case for renal cell carcinoma, a cancer known clinically to respond to anti-angiogenic therapy [29]. Both poorly and highly vascularized cancers have been shown to respond to anti-angiogenic therapy [29].

Correlation of VEGF-A expression or microvessel density with health outcomes in PDAC

VEGF-A, a potent inducer of angiogenesis, was first discovered as a secreted protein that can enhance vascular permeability [12].

Many different isoforms exist, and their different binding affinities for heparan sulfate proteogylcans (HSPGs) function to create a gradient for guiding vessels during vascular development [16]. In recent years, more insight into the alternative splicing and translation of the gene has revealed that anti-angiogenic forms and a translational read through can also be produced [30,31].

Using immunohistochemistry (IHC), several groups found that between 60 and 65% of human PDAC samples have a substantial amount of VEGF-A immunoreactivity [32–34]. In terms of gene expression, Ikeda et al. found that 27/40 (67.5%) human PDAC samples overexpress VEGFA compared to a colon cancer cell line, while Itakura et al. found a 5.2 fold increase in VEGFA expression in human PDAC samples (n = 7) compared to normal human pancreas samples (n = 4) [32,34]. More recently, by RNA-Sequencing (RNA-Seq), The Cancer Genome Atlas (TCGA) dataset shows that only 8 out of 178 (4%) human PDAC samples overexpress VEGFA, suggesting that this growth factor may not be as important in PDAC as was first surmised [8,35,36].

MVD has not been shown to be an accurate measure of angiogenesis in other cancers [29]; nonetheless, three [32–34] of four [37] studies of human PDAC samples have shown an association between VEGFA mRNA or VEGF-A protein (IHC) expression and the amount of vascularity seen in the tumor. Patients with high levels of VEGFA mRNA or VEGF-A protein (IHC) also had increased liver metastasis [33], larger tumors [34], enhanced local spread [34], and decreased survival in two [32,33] out of four [34,37] studies. Lastly, one [32] out of two [37] studies reported that increased vascularity was associated with decreased patient survival.

Pre-clinical studies targeting VEGF signaling in PDAC

Many studies have examined the potential role of targeting VEGF signaling using subcutaneous or orthotopic nude mouse models of human PDAC. Injection of human PDAC cells expressing an antisense VEGFA into the flanks of nude mice led to an 80% reduction in tumor size compared to controls [38]. When diphtheria toxin, which inhibits protein synthesis in target cells, was fused with VEGF-A to target it to the vasculature in orthotopic nude mouse models of human PDAC, it led to reduced tumor volume, tumor spread, and MVD, and improvement in survival in 1 of 2 models [39]. Injection of adenovirus vectors encoding the soluble form of the decoy receptor VEGFR-1 into subcutaneous tumor xenografts of human PDAC in SCID mice also resulted in reduced tumor growth and MVD [40]. Additionally, injection of adenovirus vectors encoding soluble VEGFR-1 or soluble VEGFR-1 plus a soluble fibroblast growth factor receptor 1 (gene: FGFR1) (FGFR-1) into subcutaneous tumor xenografts of human PDAC in nude mice resulted in reduced tumor growth [41].

The tyrosine kinase inhibitor PTK 787/ZK222584 (vatalanib) targets VEGF receptors, the platelet-derived growth factor receptors (PDGFRs), the mast/stem cell growth factor receptor Kit (gene: KIT) (SCFR), and macrophage colony-stimulating factor 1 receptor (CSF1R). Use of this compound in an orthotopic nude mouse model of human PDAC led to reduced tumor volume and MVD, and increased survival [42]. Moreover, use of VEGF-Trap (ziv-aflibercept), which is a recombinant fusion protein of the extracellular portions of VEGFR-1 and VEGFR-2 and the Fc fragment of human immunoglobulin IgG1, resulted in reduced tumor growth and MVD in subcutaneous tumor xenografts of human PDAC and reduced tumor growth and metastasis in an orthotopic nude mouse model of human PDAC [43]. These promising results provide support for the testing of anti-VEGF agents in human PDAC clinical trials.

Clinical studies in PDAC

To date, many phase II and phase III human PDAC clinical trials using different anti-angiogenic agents have been completed. Several

of these involved bevacizumab, an anti-VEGF-A monoclonal antibody, that has already been Food and Drug Administration (FDA) approved for the treatment of several other cancer types, including metastatic renal cell carcinoma in combination with interferon alpha, glioblastoma as a second-line therapy, or in combination with chemotherapy in the following cancers: platinum-resistant recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer; persistent, recurrent, or metastatic cervical cancer; metastatic colorectal cancer; or non-small cell lung cancer.

An initial Phase II trial of bevacizumab plus gemcitabine in untreated advanced PDAC patients showed a 21% objective response rate (ORR), a 6-month survival rate of 77%, and a median survival of 8.8 months (Table 1) [44]. Because these were favorable numbers compared to the pivotal trial for gemcitabine approval [45], which observed an ORR of 5%, a 6-month survival rate of 46%, and a median survival of 5.7 months, several other Phase II and Phase III studies were launched.

Several Phase II trials added bevacizumab to any existing regimen that had previously shown any sort of modest activity in PDAC. These regimens included: cisplatin and gemcitabine [46]; capecitabine and gemcitabine [47]; capecitabine, radiation, and gemcitabine [48]; oxaliplatin and gemcitabine [49]; gemcitabine and radiation [50,52], and docetaxel [51] (Table 1). However, results from the Phase III trial directly comparing bevacizumab plus gemcitabine to placebo plus gemcitabine in advanced PDAC patients showed that the addition of bevacizumab does not result in an improvement in overall survival (OS) or progression free survival (PFS) or differences in the ORR (Table 2) [61].

The difference between the Phase II and Phase III results was suggested to be due to the Phase II trial recruiting a more fit population [61]. Because such disparities are common in trials of PDAC, it was also suggested that the use of a single-arm Phase II trial is not ideal [61]. The majority of Phase II trials with other regimens were single-arm trials, and thus, most of them also concluded that the addition of bevacizumab produced questionable benefit.

In addition to VEGF-A, epidermal growth factor receptor (EGFR) and its ligands are commonly overexpressed in human PDAC, and high expression levels are also associated with worse outcomes [66–69]. The addition of cetuximab, a monoclonal antibody targeting EGFR, to gemcitabine has not led to improvements in ORRs, PFS, or OS [70], but the addition of erlotinib, a small molecule inhibitor of EGFR, to gemcitabine has been shown to provide a statistically significant improvement in survival [71]. However, the clinical relevance of this result is often questioned since the median gain in survival is only 10 days [71].

There is also evidence for EGFR's role in angiogenesis and simultaneous inhibition of EGFR and VEGFR-2 has been shown to be synergistic [66,68,72–74]. Therefore, several regimens combining cetuximab or erlotinib with bevacizumab have been tried with limited success (Table 3) [75–77]. A Phase III trial comparing bevacizumab plus erlotinib plus gemcitabine to placebo plus erlotinib plus gemcitabine in metastatic PDAC patients did not show benefit in OS, but it did show a statistically significant one month improvement in the median PFS (Table 2) [60]. Therefore, there is some rationale for using this drug combination in metastatic PDAC patients.

Additional anti-angiogenic agents that have been tried in human PDAC include axitinib, sunitinib, sorafenib, vatalanib, ziv-aflibercept, and elpamotide. The Phase II or III trial comparing axitinib, a VEGFR tyrosine kinase inhibitor, plus gemcitabine to gemcitabine alone did not provide a significant improvement in overall or PFS (Tables 1 and 2) [53,62].

Sunitinib is a small molecule tyrosine kinase inhibitor of VEGFRs, PDGFRs, and SCFR. Though a Phase III study has not been done, this molecule has been tested in the metastatic setting as either a second-line therapy [54] or a maintenance therapy in patients who did not progress after first-line chemotherapy [55]. Interestingly, in these patient groups, the drug did not do well as a second-line therapy (Table 1), but produced a statistically significant improvement in PFS compared to observation alone in the maintenance setting (hazard ratio [HR] 0.51 [95% confidence interval (CI): 0.29–0.89], p-value < 0.01) [55]. Because the duration of first-line chemotherapy is often debated due to its cumulative toxicity and unproven efficacy, sunitinib may offer an advantage in the maintenance setting.

Similarly, sorafenib is a small molecule tyrosine kinase inhibitor of serine/threonine-protein kinase B-raf (BRAF), VEGFRs, and platelet-derived growth factor receptor beta (PDGFRB) that has been tested in many different settings without benefit (Tables 1 and 3) [56–58,78]. These observations were confirmed in a Phase III trial that observed no improvement in overall or PFS upon the addition of sorafenib to gemcitabine in the treatment of advanced PDAC patients (Table 2) [63].

Vatalanib is also a multi-kinase inhibitor targeting VEGFRs, PDGFRs, SCFR, and CSF1R. In a Phase II trial, it was used as a second-line therapy in advanced PDAC patients and produced a favorable 6 month survival rate of 29% compared to historic controls (Table 1) [59]. However, it was only a single-arm trial, and with the failure of several other similar receptor tyrosine kinase inhibitors, it remains to be seen whether this drug will pan out.

Ziv-aflibercept, a recombinant fusion protein consisting of the extracellular portions of VEGFR-1 and VEGFR-2 and the Fc fragment of human immunoglobulin IgG1, is another drug that targets the VEGF pathway by trapping VEGF-A, VEGF-B, and PlGF. This drug yielded negative results in a Phase III trial compared to gemcitabine alone (Table 2) [64].

Elpamotide, a VEGFR-2 peptide, is a vaccine immunotherapy that can induce a cellular immune response against VEGFR-2 expressing endothelial cells [65,79]. In a Phase II/III trial (Table 2) of locally advanced or metastatic pancreatic cancer patients, there were no improvements in overall or PFS compared to gemcitabine alone, but a subgroup with severe injection site reactions tended to do better, suggesting that this may be a sign of immune response to the vaccine [65].

Thus, targeting the VEGF pathway alone is not an efficacious route in PDAC. Even targeting multiple players in the neoplastic process, like EGFR or other receptor tyrosine kinases, produced marginal benefit, with only two trials showing an improvement in PFS, but not OS [55,60].

Reasons for failure

The overwhelming failure of anti-angiogenic agents in the clinic leads us to speculate on the reasons for the failure. Over the last 20 years, efforts in targeting angiogenesis in cancer have focused almost entirely on the pro-angiogenic molecule VEGF-A, and there are now several FDA approved drugs for various cancers [15,18,21,22,26,80]. In reality, despite very convincing pre-clinical data, some cancers are resistant to such therapy or develop resistance over time [15,18,21,22,25,26,80]. This suggests that other angiogenic pathways that we have yet to address are involved. Indeed, other pro-angiogenic molecules include fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), angiopoietins (ANGPTs), transforming growth factor beta (gene: TGFB1) (TGF-β), and cytokines like interleukin-8 (gene: CXCL8) (IL-8) [73,81]. Thus, to block angiogenesis effectively, we need to target multiple molecules simultaneously.

Because many pro-angiogenic growth factors such as VEGF-A, FGF2, PDGFs, TGF-β, and heregulin (gene: NRG1) (HRG) bind to HSPGs to facilitate their signaling, another targetable common denominator would be these proteoglycans [73,81]. The validity of this strategy has been shown with $Kras^{LSL-G12D/+}$, $Cdkn2a^{LoxP/LoxP}$, Pdx-1-Cre (KIC) mice that were null for glypican-1 (Gpc1), one of the HSPGs. KIC mice have oncogenic Kras and deleted

Table 1
Phase II clinical trials using anti-angiogenic agents in PDAC.

Ref	Phase	Group	Drug	Experimental arm	Active comparator arm	Hazard ratio
Kindler et al. [44]	II	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + gemcitabine – ORR: 21% (11–35%) – 6 m survival: 77% (63–86%) – OS: 8.8 m (7.4–9.7 m) – PFS: 5.4 m (3.7–6.2 m)	NA	NA
Ko et al. [46]	II	Metastatic	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + cisplatin + gemcitabine – ORR: 19.2% – OS: 8.2 m (6.9–11.1 m) – TTP: 6.6 m (4.6–8.8 m)	NA	NA
Javle et al. [47]	II	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + capecitabine + gemcitabine – ORR: 22% – OS: 9.8 m (8.3–11.9 m) – PFS: 5.8 m (4.2–7.8 m)	NA	NA
Crane et al. [48]	II	Locally advanced (unresectable)	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + capecitabine + radiation followed by gemcitabine + bevacizumab – ORR: 26% – OS: 11.9 m (9.9–14 m) – PFS: 8.6 m (6.9–10.5 m)	NA	NA
Fogelman et al. [49]	II	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + oxaliplatin + gemcitabine – ORR: 36% – 6 m survival: 74% – OS: 11.9 m – PFS: 4.9 m	NA	NA
Small et al. [50]	II	Localized	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + radiation + gemcitabine, then surgery or bevacizumab + gemcitabine – ORR: 11% (4–24%) – 6 m survival: 86% – OS: 11.8 m – PFS: 9.9 m	NA	NA
Astsaturov et al. [51]	II	Metastatic	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab – ORR: 0% – OS: 165 d – PFS: 43 d	• Bevacizumab + docetaxel – ORR: 0% – OS: 125 d – PFS: 48 d	NA
Van Buren II et al. [52]	II	Localized (potentially resectable)	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Neoadjuvant bevacizumab + gemcitabine, then radiation – OS: 16.8 m (14.9–21.3 m) – PFS: 6.6 m (4.9–12.4 m)	NA	NA

Table 1 (*continued*)

Ref	Phase	Group	Drug	Experimental arm	Active comparator arm	Hazard ratio
Spano et al. [53]	II	Advanced	Axitinib (SMI of VEGFRs)	• Axitinib + gemcitabine – ORR: 7% (2.4–16.1%) – OS: 6.9 m (5.3–10.1 m) – PFS: 4.2 m (3.6–10.2 m)	• Gemcitabine – ORR: 3% (0.1–15.3%) – OS: 5.6 (3.9–8.8) m – PFS: 3.7 (2.2–6.7) m	• OS HR 0.71 (0.44–1.13) • PFS HR 0.79 (0.43–1.45)
O'Reilly et al. [54]	II	Metastatic (second-line therapy)	Sunitinib (SMI of VEGFRs, PDGFRs, SCFR)	• Sunitinib – ORR: 1.4% – OS: 3.68 m (3.06–4.24 m) – PFS: 1.31 m (1.25–1.38 m)	NA	NA
Reni et al. [55]	II	Metastatic (maintenance therapy)	Sunitinib (SMI of VEGFRs, PDGFRs, SCFR)	• Sunitinib – ORR: 0% – OS: 10.6 m (6.2–18.9 m) – PFS: 3.2 m	• Observation – ORR: 0% – OS: 9.2 m (5.9–16.3 m) – PFS: 2 m	• OS HR 0.11 (0.4–1.26) • PFS HR 0.51* (0.29–0.89)
El-Khoueiry et al. [56]	II	Metastatic	Sorafenib (SMI of BRAF, VEGFR-2, PDGFRB)	• Sorafenib – 6 m survival: 43% – OS: 4.3 m (3.3–8.3 m) – PFS: 2.3 m (1.2–5.7 m)	• Sorafenib + gemcitabine – 6 m survival: 53% – OS: 6.5 m (5.5–8 m) – PFS: 2.9 m (2.1–4.3 m)	NA
Kindler et al. [57]	II	Advanced	Sorafenib (SMI of BRAF, VEGFR-2, PDGFRB)	• Sorafenib + gemcitabine – ORR: 0% – 6 m survival: 23% (6–47%) – OS: 4 m (3.4–5.9 m) – PFS: 3.2 m (1.6–3.6 m)	NA	NA
Cascinu et al. [58]	II	Advanced	Sorafenib (SMI of BRAF, VEGFR-2, PDGFRB)	• Sorafenib + cisplatin + gemcitabine – ORR: 3.4% – OS: 7.5 m (5.6–9.7 m) – PFS: 4.3 m (2.7–6.5 m)	• Cisplatin + gemcitabine – ORR: 3.6% – OS: 8.3 m (6.2–8.7 m) – PFS: 4.5 m (2.5–5.2 m)	• OS HR 0.95 (0.62–1.48) • PFS HR 0.92 (0.62–1.35)
Dragovich et al. [59]	II	Advanced (second-line therapy)	Vatalanib (SMI of VEGFRs, PDGFRs, SCFR, CSF1R)	• Vatalanib – ORR: 3.1% – 6 m survival: 29% (18–41%) – PFS: 2 m	NA	NA

Ref, reference; SMI, small molecule inhibitor; ORR, objective response rate; OS, overall survival; PFS, progression free survival; TTP, time to progression; HR, hazard ratio; m, month(s); d, days(s); VEGF-A, vascular endothelial growth factor A (gene: VEGFA); VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; SCFR, mast/stem cell growth factor receptor Kit (gene: KIT); BRAF, serine/threonine-protein kinase B-raf; VEGFR-2, vascular endothelial growth factor receptor 2 (gene: KDR); PDGFRB, platelet-derived growth factor receptor beta; CSF1R, macrophage colony-stimulating factor 1 receptor.
Numbers that appear in parentheses represent the 95% confidence interval.
* Statistically significant.

Table 2
Phase III clinical trials using anti-angiogenic agents in PDAC.

Ref	Phase	Group	Drug	Experimental arm	Active comparator arm	Hazard ratio
Van Cutsem et al. [60]	III	Metastatic	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + erlotinib + gemcitabine – ORR: 13.5% (9.8–17.9%) – OS: 7.1 m (0–19.8 m) – PFS: 4.6 m (0–18.3 m)	• Placebo + erlotinib + gemcitabine – ORR: 8.6% (5.6–12.4%) – OS: 6 m (0.1–19.5 m) – PFS: 3.6 m (0–13.6 m)	• OS HR 0.89 (0.74–1.07) • PFS HR 0.73* (0.61–0.86)
Kindler et al. [61]	III	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + gemcitabine – ORR: 13% – OS: 5.8 m (4.9–6.6 m) – PFS: 3.8 m (3.4–4 m)	• Placebo + gemcitabine – ORR: 10% – OS: 5.9 m (5.1–6.9 m) – PFS: 2.9 m (2.4–3.7 m)	• OS HR 1.044 (0.88–1.24)
Kindler et al. [62]	III	Advanced	Axitinib (SMI of VEGFRs)	• Axitinib + gemcitabine – ORR: 5% (2.5–8.3%) – OS: 8.5 m (6.9–9.5 m) – PFS: 4.4 m (4–5.6)	• Placebo + gemcitabine – ORR: 2% (0.4–4%) – OS: 8.3 m (6.9–10.3) m – PFS: 4.4 m (3.7–5.2) m	• OS HR 1.014 (0.786–1.309) • PFS HR 1.006 (0.779–1.298)
Gonçalves et al. [63]	III	Advanced	Sorafenib (SMI of BRAF, VEGFR-2, PDGFRB)	• Sorafenib + gemcitabine – ORR: 23% – OS: 8 m (6–10.8 m) – PFS: 3.8 m (3.1–6 m)	• Placebo + gemcitabine – ORR: 19% – OS: 9.2 m (7.7–11.6 m) – PFS: 5.7 m (3.7–7.5 m)	• OS HR 1.27 (0.837–1.932) • PFS HR 1.04 (0.697–1.545)
Rougier et al. [64]	III	Advanced	Ziv-Aflibercept (recombinant fusion protein that traps VEGF-A, VEGF-B, PlGF)	• Ziv-aflibercept + gemcitabine – 6 m survival: 54% (47–61%) – OS: 6.5 m (5.6–7.9 m) – PFS: 3.7 m (3.5–4.5 m)	• Placebo + gemcitabine – 6 m survival: 63% (56–69%) – OS: 7.8 m (6.8–8.6 m) – PFS: 3.7 m (3.5–4.6 m)	• OS HR 1.165 (0.921–1.473) • PFS HR 1.018 (0.828–1.253)
Yamaue et al. [65]	III	Advanced or metastatic	Elpamotide (epitope peptide of VEGFR-2)	• Elpamotide + gemcitabine – OS: 8.36 m (7.46–10.18 m) – PFS: 3.71 m (2.10–3.98 m)	• Placebo + gemcitabine – OS: 8.54 m (7.33–10.84 m) – PFS: 3.75 m (2.27–5.59 m)	• OS HR 0.87 (0.486–1.557)

Ref, reference; SMI, small molecule inhibitor; ORR, objective response rate; OS, overall survival; PFS, progression free survival; HR, hazard ratio; m, month(s); VEGF-A, vascular endothelial growth factor A (gene: VEGFA); VEGFR, vascular endothelial growth factor receptor; BRAF, serine/threonine-protein kinase B-raf; VEGFR-2, vascular endothelial growth factor receptor 2 (gene: KDR); PDGFRB, platelet-derived growth factor receptor beta; VEGF-B, vascular endothelial growth factor B (gene: VEGFB); PlGF, placenta growth factor (gene: PGF).
Numbers that appear in parentheses represent the 95% confidence interval.
 * Statistically significant.

cyclin-dependent kinase inhibitor 2A (Cdkn2a), which encodes for the p16^{INK4a} cell cycle inhibitor and the p19^{Arf} tumor suppressor, in the pancreas due to Cre-mediated recombination. KIC mice null for Gpc1 showed attenuated tumor growth, progression, and invasiveness, and decreased expression of pro-angiogenic genes compared to KIC mice that were wild type for Gpc1 [82].

Another major contributor to the lack of efficacy is the fact that drug delivery in PDAC is impaired due to high interstitial pressures and collapsed vessels [28]. It is possible that efficacy could be improved if anti-angiogenic therapy was administered simultaneously with a stromal depleting agent known to increase perfusion. Out of three recent pre-clinical studies that depleted various components of the stroma, two resulted in improved perfusion [27,83,84], while only one did not cause other untoward effects [28,85]. This was the study that utilized recombinant hyaluronidase (PEGPH20) to deplete the stroma, an agent now fast-tracked by the FDA to be used as an investigative therapy in combination with gemcitabine and nab-paclitaxel for the treatment of patients with metastatic pancreatic cancer [28,85]. Initial Phase II results combining PEGPH20 with nab-paclitaxel/gemcitabine have shown a statistically significant doubling of the ORR, with a trend toward improved PFS and OS in patients with high levels of hyaluronan [86]. Another strategy to promote better drug delivery would be to normalize the vasculature via stromal remodeling instead of depletion [87], or via vascular promotion, a

Table 3
Phase II clinical trials using an anti-angiogenic agent + EGFR inhibitor in PDAC.

Ref	Phase	Group	Drug	Experimental arm	Active comparator arm	Hazard ratio
Ko et al. [75]	II	Metastatic	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + erlotinib – ORR: 3% – 6 m survival: 22% – OS: 102 d (74–117 d) – TTP: 40 d (35–41 d)	NA	NA
Ko et al. [76]	II	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + cetuximab – ORR: 3.4% (0.1–17.8%) – 6 m survival: 41.4% (23.7–58.3%) – OS: 4.17 m (2.69–8.74) – PFS: 1.91 m (1.81–2.76 m)	• Bevacizumab + cetuximab + gemcitabine – ORR: 13.8% (3.9–31.7%) – 6 m survival: 39.3% (21.7–56.5%) – OS: 5.41 m (3.84–6.74 m) – PFS: 3.55 m (2–5.59 m)	NA
Watkins et al. [77]	II	Advanced	Bevacizumab (anti-VEGF-A monoclonal antibody)	• Bevacizumab + erlotinib + capecitabine + gemcitabine – ORR: 23% (11–38%) – OS: 12.6 m – PFS: 8.4 m	NA	NA
Cardin et al. [78]	II	Advanced	Sorafenib (SMI of BRAF, VEGFR-2, PDGFRB)	• Sorafenib + erlotinib – OS: 3.3 m or 99.5 d (71–188 d)	NA	NA

Ref, reference; SMI, small molecule inhibitor; ORR, objective response rate; OS, overall survival; PFS, progression free survival; TTP, time to progression; HR, hazard ratio; m, month(s); d, days(s); EGFR, epidermal growth factor receptor; VEGF-A, vascular endothelial growth factor A (gene: VEGFA); BRAF, serine/threonine-protein kinase B-raf; VEGFR-2, vascular endothelial growth factor receptor 2 (gene: KDR); PDGFRB, platelet-derived growth factor receptor beta.
Numbers that appear in parentheses represent the 95% confidence interval.

mechanism which involves administering agents that enhance angiogenesis, flow, and the leakiness of vessels [88].

Additionally, it has been shown that the tumor microenvironment of transplantable models is not the same as that seen in a genetically engineered mouse model (GEMM) [27]. In the transplantable models, there is a lack of stroma and the pancreatic cancer cells are close to the vessels [27]. For that reason, many cytotoxic agents that were shown to be ineffective in human trials initially showed efficacy when tested in xenograft models [27,89]. Later, it was found that such agents were just as ineffective when used in GEMMs [27,89]. It is perhaps the same story with the anti-angiogenic agents, as they were primarily only tested in subcutaneous or orthotopic nude mouse models of human PDAC. Future studies should also utilize the increasing number of available GEMMs for PDAC [90,91].

As is often observed in many clinical trials, patient responses are variable, with only a subset of patients benefiting from the therapy, while overall, no positive effect may be seen. It would be useful if we could identify those patients who might benefit the most via the use of predictive biomarkers. Though some trials have attempted to look for correlations between certain known pro-angiogenic molecules circulating in the plasma and treatment response, none have been successful to date [44,46,51,59]. With an increasing number of studies utilizing high throughput technologies like RNA-Seq to profile human tumors, it is possible that a gene expression signature could be used. In fact, we have already identified such a signature by using TCGA RNA-Seq data [92–93].

Because most approved indications for bevacizumab involve concomitant administration with some form of cytotoxic chemotherapy, at least one clinical study suggested that even if bevacizumab was effective at normalizing the vasculature sufficiently to improve drug delivery, the fact still remains that we lack any effective chemotherapeutic or targeted agent for the treatment of PDAC [61].

In summary, future studies of angiogenesis in PDAC should consider potential resistance mechanisms to targeted therapies, use appropriate pre-clinical models that can recapitulate the microenvironment seen in human PDAC, and use biomarkers or gene signatures to select patients for clinical trials.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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