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Does Axl have potential as a therapeutic target in pancreatic cancer?

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Abstract

Introduction: Pancreatic cancer is a leading cause of cancer-related death. Metastasis, therapy resistance, and immunosuppression are dominant characteristics of pancreatic tumors. Strategies that enhance the efficacy of standard of care and/or immune therapy are likely the most efficient route to improve overall survival in this disease.

Areas covered: Axl, a member of the TAM (Tyro3, Axl, MerTK) family of receptor tyrosine kinases, is involved in cell plasticity, chemoresistance, immune suppression, and metastasis in various cancers, including pancreatic cancer. This review provides an overview of Axl and its function in normal conditions, summarizes the regulation and function of Axl in cancer, and highlights the contribution of Axl to pancreatic cancer as well as its potential as a therapeutic target.

Expert opinion: Axl is an attractive therapeutic target in pancreatic cancer because it contributes to many of the roadblocks that hamper therapeutic efficacy. Clinical evidence supporting Axl inhibition in pancreatic cancer is currently limited; however, multiple clinical trials have been initiated or are in the planning phase to test the effect of inhibiting Axl in conjunction with standard therapy in pancreatic cancer patients. We anticipate that these studies will provide robust validation of Axl as a therapeutic target in pancreatic cancer.

Keywords

Axl; immunosuppression; metastasis; pancreatic cancer; therapy resistance; epithelial plasticity

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1. Introduction

Pancreatic cancer is the third-leading cause of cancer-related death in the US [1]. Three dominant characteristics of pancreatic cancer are metastasis, therapy resistance, and immunosuppression [2]. Axl, a member of the TAM (Tyro3, Axl, MerTK) family of receptor tyrosine kinases (RTKs), is involved in cell plasticity, chemoresistance, immune suppression, and metastasis in many cancers, including pancreatic cancer. This review provides an overview of Axl and its function in normal biology, summarizes the regulation and function of Axl in cancer, and highlights the contribution of Axl to pancreatic cancer as well as its potential as a therapeutic target.

2. The structure of Axl and its ligand

Axl (anexelekto, uncontrolled), also known as UFO, was first identified as an RTK in chronic myelogenous leukemia patients in 1991 [3]. The *Axl* locus is on chromosome 19q13.2 and includes 20 exons. Exons 1–10 encode a signal peptide, two immunoglobulin (Ig) domains and two fibronectin type III domains, which form the major extracellular domains of the Axl protein. Exon 11 encodes a short extracellular proteolytic cleavage site and a single-pass transmembrane domain. Exons 12–20 encode the intracellular domain, which includes the tyrosine kinase domain. There are two distinct Axl transcripts, differing by the presence or absence of exon 10 by alternative splicing [3]. Full-length Axl protein contains 894 amino acids and has an expected molecular weight of 98 kDa. However, due to posttranslational glycosylation, the actual Axl protein is 120 kDa (partial glycosylation) or 140 kDa (complete glycosylation) [4]. Axl can be cleaved at the proteolytic cleavage site (VKEPSTPAFSWPWW) in exon 11, resulting in an 80 kDa soluble protein containing only the extracellular domain [4]. The level of soluble Axl in serum has potential as a diagnostic marker for different diseases such as hepatocellular carcinoma [5], intracranial aneurysm rupture [6], acute coronary syndrome [7], abdominal aortic aneurysms [8], liver fibrosis and cirrhosis [9], sepsis, systemic inflammatory response syndrome [10], and systemic lupus erythematosus [11].

As a member of the TAM RTK family, Axl shares a similar protein structure with its family members MerTK and Tyro3 [12]. There are two main ligands for TAM receptors, growth arrest-specific gene 6 (Gas6) and protein S (PROS). In addition, novel ligands, such as Tubby, Tulp-1 [13], and Galectin-3 [14], have also been discovered. Gas6 has the highest affinity for and is the major ligand for Axl [15], although recent data suggest that PROS can function as an activating Axl ligand in mesenchymal glioma sphere cultures [16]. Gas6 is a 75 kDa protein with a γ -carboxyglutamate [17] domain, four epidermal growth factor (EGF)-like repeats, and two laminin G (LG)-like domains. The vitamin K-dependent γ -carboxylation of glutamate to Gla in Gas6 is required for its binding to phosphatidylserine (PS) [15]. Once the LG domain of Gas6 binds to the Ig domain of Axl, it induces the dimerization of the Gas6/Axl binding complex. Ligand-induced activation of Axl occurs only after the Gla domain of Gas6 binds to PS [15] (Figure 1). Upon Gas6 stimulation, several tyrosine residues of Axl (e.g., Y702, Y779, Y821, and Y866) get autophosphorylated and function as docking sites for multiple substrates, including the P85 subunit of phosphatidylinositol-3-kinases (PI3K), growth factor receptor-bound protein 2,

phospholipase C, and c-Src [18, 19]. Elmo is another substrate of Axl and is required for Axl-induced Rac activation [20]. Besides the canonical ligand-dependent activation, Axl can also be activated by forming heterodimers with non-TAM family proteins (such as epidermal growth factor receptor [EGFR], platelet-derived growth factor receptors, or MET) or a TAM family member (MerTK or Tyro3) [21].

3. The function of Axl in normal basal condition

3.1 Cell survival, proliferation, and migration

As early as 1996, Axl was shown to affect mitogenic and survival activities for serum-starved NIH3T3 fibroblasts. Goruppi et al. [22] demonstrated that Gas6 can selectively induce entry into S phase in Axl-expressing cells through the PI3K/Akt pathway and Src. Axl was also shown to promote NIH3T3 fibroblast survival by protecting serum-deprived cells from apoptosis [23]. Overexpression of Axl in NIH3T3 cells has also been reported to lead to neoplastic transformation [3]. The Gas6/Axl pathway also promotes cell survival, proliferation, and growth in other cell types, such as Schwann cells [24, 25], mesangial cells [26], chondrocytes [27], and lens epithelial cells [28]. In GnRH neurons, Axl was shown to inhibit apoptosis via Erk and PI3K/Akt pathways and mediate neuronal migration through p38 or Ras and Rac [29, 30, 31].

3.2 Angiogenesis

The Gas6/Axl pathway can mediate survival in endothelial cells through NF- κ B by activating anti-apoptotic protein Bcl-2 and inhibiting pro-apoptotic protein caspase 3 [32]. Axl also supports vascular smooth muscle cell (VSMC) migration and chemotaxis, mainly through the PI3K pathway [33]. Axl-deficient mice have been exploited to investigate the contribution of Axl to various pathways. For example, Axl-deficient mice showed reduced responses to VEGF-A-induced permeability and angiogenesis, suggesting that Axl activity on endothelial cells is critical for VEGF-A function in normal endothelial cells [34]. Furthermore, Axl expression is induced in endothelial cells and VSMCs by vascular injury, where it functions to protect cells from apoptosis [35].

3.3 Efferocytosis

Apoptosis and the clearance of apoptotic cells (a process called efferocytosis) are essential for development and tissue homeostasis. Apoptotic cells display an “eat me” signal, such as PS, which is recognized by PS receptors (including the TAM receptors) expressed on immune cells (dendritic cells [DCs] and macrophages) to induce phagocytic engulfment [36]. PS is a negatively charged phospholipid found on the inner leaflet of the plasma membrane of all eukaryotic cells. Upon the induction of apoptosis, PS is flipped to the outer leaflet of the plasma membrane, where it stimulates efferocytosis. Under certain circumstances, viable cells can expose PS. For example, in the tumor microenvironment, endothelial cells and tumor cells often externalize PS due to stressful conditions (hypoxia, cytokines, and oxidative stress) [36]. As mentioned above, PS is required for Gas6-mediated Axl activation. Thus, it is not surprising that Axl signaling in DCs or macrophages is involved in efferocytosis. In 2007, Seitz et al. [37] reported a comprehensive study on the contribution of the three TAM family members to efferocytosis in different phagocytic cell

types. They found that macrophages from *Axl*^{-/-} mice were 50% less able to phagocytose apoptotic cells compared to macrophages from wild-type (WT) mice, whereas MerTK elimination almost abolished macrophage-mediated efferocytosis. On the other hand, DCs relied mostly on Axl and Tyro3 to clear apoptotic cells [37]. Phagocytosis mediated by macrophages and DCs is critical in inflammatory diseases caused by abnormal efferocytosis. For instance, Axl is expressed highly on airway macrophages in healthy humans and can be potently induced by granulocyte-macrophage colony-stimulating factor, poly(I:C), and type I interferon (IFN) [38]. Patients with moderate-to-severe asthma have a reduced expression of Axl in airway macrophages, suggesting an association of Axl expression with the efficiency of apoptotic cell removal in airways [39]. This conclusion is further supported by a study showing that the number of apoptotic cells was elevated in the airways of *Axl*^{-/-} mice infected with influenza [38].

3.4 Immune response

Besides DCs and macrophages, Axl is also expressed on other immune cells, including regulatory T cells (Tregs) and natural killer (NK) cells [40, 41, 42]. In general, the expression of Axl can be elevated by proinflammatory stimuli, such as toll-like receptor (TLR)-dependent stimuli (LPS and poly(I:C)), and IFN- γ , suggesting that Axl can be an inflammatory response receptor [43]. In DCs, Axl expression is elevated by the TLR-induced IFNAR-STAT1 pathway, which in turn induces a suppressor of cytokine signaling (SOCS1 and SOCS3), an intrinsic feedback inhibitor of the inflammatory response [44]. Axl also suppresses inflammatory cytokine production in macrophages by inducing the transcriptional suppressor Twist to bind to the tumor necrosis factor α promoter [45]. Additionally, Gas6/Axl signaling in CD4⁺ CD25⁺ Tregs increases the expression of Foxp3 and CTLA-4 and promotes immunosuppressive activity [42]. Another important function of Axl in the immune response is regulating NK cell development. Axl inhibition can diminish interleukin-15 (IL-15)-induced development of NK cells from CD34⁺ hematopoietic progenitor cells [40]. This regulation is probably through the regulatory effect of Axl on c-Kit signaling or fibromyalgia syndrome-like tyrosine kinase 3 (FLT3) signaling [40].

3.5 Viral infection

Gas6 has been implicated in the infectivity of enveloped viruses, which externalize PS. Gas6 binds the exposed PS and can mediate the binding of the virus to target cells via interaction with TAM receptors, including Axl. In fact, through this mechanism, Axl has been reported to be involved in the infection of multiple viruses, including Zika virus [46, 47] and Zaire Ebola virus [48]. In addition to using Axl as an entry point into cells, the activation of Axl on immune cells such as DCs inhibits type I IFN signaling, providing an indirect mechanism that promotes viral infection [49].

3.6 Other functions

Axl is also important in platelet aggregation and fibrosis. The Gas6/Axl/PI3K pathway can amplify outside-in integrin α IIb β 3 signaling [50] and increase stabilization of platelet aggregation [50]. Thus blocking Gas6/Axl/ α IIb β 3 signaling has potential as an approach for thrombosis prevention that might be especially beneficial in the context of cancer. In addition, Axl has been implicated in the migration and proliferation of lung fibroblasts

associated with idiopathic pulmonary fibrosis. For example, Espindola et al. [51] recently found that the inhibition of Axl or loss of Gas6 decreased fibrosis in mouse models of lung injury. Axl has also been implicated in fibrosis associated with chronic liver disease. Barcena et al. [52] stimulated hepatic stellate cells (HSCs) from WT and *Axl*^{-/-} mice with Gas6 and found that Axl-deficient HSCs had a significant reduction in markers of HSC activation. Consistently, the authors observed that chronic damage (CCl4 administration) resulted in decreased liver fibrogenesis in *Axl*^{-/-} mice [52]. These studies indicate that Axl inhibition has potential as a therapeutic tool for fibrotic indications.

4. Axl expression and regulation in cancer

Axl is expressed highly in primary tumors and metastases compared to normal tissue [53]. High Axl expression and activity is associated with poor prognosis, metastasis, and chemoresistance in multiple types of cancers, including pancreatic cancer [21, 54]. Elevated Axl expression in tumors is induced by transcriptional, translational, and posttranslational modification [55]. However, alteration of the *Axl* gene was found in 3% (34/1088) of sequenced pancreatic cancer patients, of which 2.3% of cases showed copy number amplification with the rest being missense mutations [56, 57]. These data support that the majority of high Axl activity in tumors is due to elevated expression and not a result of activating mutations or genomic alteration.

The first study [58] characterizing the promoter and cis-acting elements of Axl showed that a GC-rich region upstream of the translational start site is sufficient for basal Axl promoter activity. This GC-rich region contains five specificity protein (Sp)-binding sites that can be bound by Sp1 and Sp3, and is controlled by CpG island methylation [58]. This promoter also harbors multiple myeloid zinc finger gene-1 (MZF1) binding motifs. MZF1 can bind to these motifs and activate Axl promoter activity [59]. Another transcription factor family, activator protein 1 (AP-1), has members that can bind to Axl promoter and are controlled by protein kinase C (PKC)/extracellular signal-regulated kinase 1/2 (ERK1/2), or PKC/p-38 [60]. Mutant p53 can also bind to Axl promoter where it has been shown to induce histone acetylation and recruit additional transcriptional factors that drive increased Axl expression [61]. In addition, the Axl promoter is a direct target of HIF1/2 [62]. Thus, hypoxia is likely a major mechanism of elevated Axl expression in solid tumors. Besides transcriptional regulation, the translation of Axl can be reduced by microRNAs, including miR-34a and miR-199a/b [63], through targeting the 3'-UTR of Axl. HSP90 is also an important posttranslational regulator of Axl. HSP90 protects Axl from ubiquitination and proteasomal degradation and also facilitates the glycosylation and translocation of Axl to the cell membrane [64].

5. The function of Axl in cancer

Axl makes pleiotropic contributions to the development and progression of cancer. It can promote cancer cell survival, proliferation, migration, invasion, and cancer cell stemness and can inhibit apoptosis in various cancer cell lines [17, 65, 66]. It also affects the tumor microenvironment and contributes to immunosuppression [67, 68, 69]. Axl has also been shown to regulate the expression of miR-374a and miR-548b in cancer cells, miRs that are

associated with cell proliferation, EMT, and tumor cell migration [70]. Much of our understanding of Axl has come from inhibition studies via the genetic inhibition of Axl, through siRNA, shRNA, and CRISPR-Cas9-mediated ablation or pharmacologic inhibition strategies. Pharmacologic strategies include blocking Gas6 or Axl with monoclonal antibodies (mAbs) and small-molecule inhibitors, and some of the strategies have already begun clinical testing [71]. Axl inhibition can suppress tumor growth and metastasis, prolong animal survival, and sensitize tumors to standard therapies in different cancer models. The major functions of Axl in cancer are introduced in detail below.

5.1 Cell plasticity and metastasis

The most studied function of Axl in cancer is its association with metastasis. Metastasis is the most common cause of cancer-related death. Briefly, metastasis of epithelial tumors requires that cancer cells delaminate from epithelial nests, invade the surrounding stroma and migrate to and intravasate into blood or lymphatic vessels. Once in the circulation, metastatic tumor cells extravasate out of vessels into a target organ where successful cells form micrometastases that might progress to macrometastases [72]. This complex process is facilitated by tumor cell epithelial plasticity, a transcriptionally regulated program akin to the developmental process of epithelial-to-mesenchymal transition (EMT). Epithelial plasticity enables tumor cells to adopt mesenchymal-like characteristics that facilitate tumor cell invasion, migration, and resistance to stressful conditions, thereby enhancing the efficiency of metastasis [73, 74].

Axl is highly associated with epithelial plasticity, cell invasiveness, and tumor metastasis. This is highlighted by work from Gjerdrum et al., [17] who used a 3D matrigel culture system to show that shRNA-mediated knockdown of Axl in human breast cancer cells (MDA-MB-231) resulted in small, round colonies while the parental cell line generated large colonies with stellate invasive cell projections. These studies suggested that Axl is required for breast cancer cell invasiveness, which was consistent with their in vivo studies that demonstrated that the loss of Axl substantially reduced metastasis in vivo [17]. Axl has also been shown to be critical for intravasation, extravasation, and colonization of tumor cells in target organs. Goyette et al. [75] injected luciferase labelled Axl knockout (KO) and WT breast cancer cells into the mammary fat pads of NOD-SCID mice, and quantified circulating tumor cells (CTCs) when primary tumors reached a predetermined size. They found that after Axl deletion, there was a significant reduction ($p = 0.0057$) of CTCs in the blood compared to parental cells, indicating the importance of Axl in tumor cell intravasation. They also used the same cells for i.v. injection studies, thereby bypassing the challenge of intravasation, and found again that Axl depletion resulted in significantly fewer ($p < 0.01$) metastatic lesions, suggesting that Axl-null cells had a decreased ability to exit blood vessels and invade into the lung. Finally, they generated cancer cells with an inducible shRNA against Axl and knocked down Axl after cells had already extravasated into the lungs and again they found that the loss of Axl resulted in reduced lung metastases ($p < 0.05$) [75].

The mechanisms of how Axl regulates cell plasticity have been investigated by numerous groups (Figure 2A). There is evidence that Axl is a downstream effector of TGF β and is

required for the maintenance of an EMT-like phenotype in tumor cells. This is supported by the findings that Axl expression is elevated by TGF β and EMT transcription factors Snail and Slug [76]. Further, Axl inhibition in cancer cells can abolish TGF β -induced invasion, an effect that can be rescued by re-expression of Axl [17]. Axl in turn regulates the expression of EMT markers through the PI3K/Akt pathway [77]. Additionally, the Gas6/Axl pathway can induce epithelial plasticity as evidenced by Gas6/Axl induction of EMT transcription factors such as Slug and mesenchymal markers including N-cadherin and vimentin [78]. Furthermore, ligand-induced Axl activation can enhance migration, invasion, and colony formation in prostate cancer cells [79] and breast cancer stem cells [65]. Other mechanisms of Axl-mediated metastasis include increased expression of matrix metalloproteinases (MMPs) [53, 80, 81, 82] and proteins involved in cytoskeletal dynamics such as Rac [20]. Axl has also been implicated in establishing a favorable microenvironment for metastatic colonization. Thrombospondin 2 secreted by Axl-positive mesenchymal state metastatic initiating cells can activate lung fibroblasts in breast cancer models. The activated fibroblasts subsequently shift cancer cells into a proliferation-compatible state in the lung metastatic site [67].

5.2 Therapy resistance

High expression of Axl has been found in therapy-resistant patients with multiple cancers. Axl is also elevated in cancer cells after therapy, which can result in acquired therapy resistance [83, 84, 85] (Figure 2B). For example, chemotherapy has been shown to increase the expression of Axl by reducing methylation at the Axl promoter [83]. Reactive oxygen species (ROS) induced by chemotherapy can also stimulate Axl activity [85]. Axl has also been implicated in the development of resistance to targeted therapies. The most notable example is Axl-mediated resistance to EGFR inhibitors in lung cancer where Axl appears to compensate for the loss of EGFR-mediated tumor cell survival. A plausible explanation is that under therapy stress, cancer cells induce epithelial plasticity, which results in resistance due to changes in drug uptake, reduced tumor cell proliferation, and increased apoptosis. This is supported by the findings that there is increased activation of Axl and expression of the mesenchymal marker vimentin in EGFR-mutant lung cancer patients with acquired resistance to EGFR-targeted therapies [21]. Gene expression and proteomic and drug response analysis in non-small-cell lung cancer (NSCLC) also revealed that mesenchymal cells showed greater resistance to EGFR inhibitors, PI3K/Akt inhibitors, and chemotherapies, with *Axl* as one of the key genes in the mesenchymal signature [86, 87]. A follow-up study showed that Axl inhibition resulted in the dysfunction of homologous recombination DNA repair pathways and sensitized cells to PARP inhibition [88]. Additionally, nuclear translocation of EGFR, a mechanism that cancer cells use to bypass the inhibitory effects of EGFR-targeted therapies, is stimulated by Axl through SKI and NRG1 [89]. Axl is a key regulator of cancer cell stemness in multiple types of cancers, including breast cancer [90] and glioblastoma [91], where it promotes therapy resistance through maintaining cancer stem cell properties. In cutaneous squamous cell carcinoma, Axl expression correlates with the expression of putative cancer stem cell markers CD44 and ALDH1 as well as increased resistance to chemotherapy [92]. Axl inhibition can also enhance the efficacy of therapies independent of EMT. For example, Wilson et al. [93]

showed that Axl sensitizes cancer cells to antimitotic agents, through the regulation of CDC2 dephosphorylation, a cell cycle-dependent kinase that controls mitotic entry.

5.3 Immunosuppression

Nonresponsiveness to T-cell immunotherapy and anti-PD1 therapy is connected to a plasticity gene signature that is associated with increased Axl expression, suggesting that Axl participates in immunotherapy resistance and immunosuppression regulation [68, 94] (Figure 2C). This is not unreasonable given the function of Axl on immune cells described above and the fact that Axl signaling on tumor cells can induce the expression of PD-L1 via the PI3K/Akt pathway [95]. Furthermore, CRISPR-Cas9 KO of Axl in breast cancer cells resulted in increased MHC-I expression via STAT1 [94]. Additionally, Axl has been implicated in mediating cross-talk between tumor cells and tumor-associated immune cells. For example, analysis of cytokine and chemokines in conditioned media from Axl WT and KO breast cancer cells showed changes in myeloid-supporting cytokines, macrophage-recruiting chemokines, and NF- κ B target cytokines [96]. Furthermore, conditioned medium from cancer cells with activated Axl signaling can polarize tumor-associated macrophages to an M2 phenotype with increased expression of immunosuppressive cytokines and chemokines [97]. Additionally, Axl KO orthotopic breast tumors had more myeloid DCs, which interacted with the increased MHC-I on the tumor cells, and proliferating T cells, which produced TNF- α and IFN- γ [68]. Further evidence supporting Axl in immune regulation comes from the observations that the blockade of Axl on immune cells can result in a proinflammatory profile and a reduction of tumor cell metastasis and drug resistance. This is illustrated by a fascinating study by Paolino et al. [41], who found that TAM receptors are ubiquitination substrates for casitas B-lineage lymphoma proto-oncogene-b (Cbl-b) in NK cells, where the inhibition of TAM receptors enhances the antimetastatic activity of NK cells. Finally, Guo Z et al. [95] showed that pharmacological inhibition of Axl resulted in increased infiltration of total leukocytes, CD4⁺ T cells, CD8⁺ T cells, and cDCs and a decrease in macrophages in a breast cancer model. The recruited T cells had a high level of CD69, Ki67, and IFN- γ , indicating their proliferation, activation, and effector function. The expression of immunosuppressive cytokines, such as Arginase 1, TGF- β , and IL-10, was also decreased. Importantly, the antitumor efficacy of Axl inhibition was reduced by the depletion of CD4⁺ and CD8⁺ T cells or NK cells or the use of nude mice. These data in aggregate strongly support that Axl contributes directly to the immunosuppressive microenvironment of cancer [95].

6. The function of Axl in pancreatic cancer

The first report demonstrating the functional significance of Axl in pancreatic cancer was published by the Maitra group in 2009 [53]. They used immunohistochemical analysis of Axl in tumors from 99 pancreatic cancer patients. Axl expression was found in 54 (55%) of the tumors where the protein was localized to the cytoplasm of neoplastic cells, with minimal to no expression in normal pancreatic ductal epithelium. They also demonstrated that in these patients, Axl protein expression was significantly associated with lymph node metastases and significantly shorter median survival than in patients with Axl-negative tumors. The investigators also showed that three out of five pancreatic cancer cell lines had

high Axl expression compared to normal human ductal pancreatic epithelial (HDPE) cells and that shRNA-mediated knockdown of Axl inhibited cell viability, anchorage-independent growth, migration, and invasion in MiaPaCa-2 cells. Axl knockdown also resulted in a morphology change of MiaPaCa-2 cells with a loss of organized polarity and well-formed filopodia. These phenotype changes were accompanied by the inhibition of multiple pathways, including MAPK/Erk and PI3K/Akt, and a reduction of activated Rho family GTPases, which control cytoskeletal dynamics as well as the EMT transcription factors Snail, Slug, and Twist, and MMP-9. Two additional independent studies have subsequently assessed Axl expression in clinical samples from pancreatic cancer patients and confirmed that Axl expression correlates with worse outcome [54, 98]. In the two studies, 70% and 76% of patients had tumor tissues with high Axl levels, which was associated with worse prognosis, distant metastasis, and poor overall survival and recurrence-free survival. Song et al. [54] identified that Axl was expressed highly in 9 out of 12 pancreatic cancer cell lines compared to HDPE cells. They also demonstrated that Axl silencing by shRNA in Panc-28 and Panc-1 cells decreased cell invasion, Akt activation, and increased radiation-induced PARP cleavage and apoptosis [54]. Proteomic analysis of pancreatic cancer patient-derived cell lines revealed that Axl was hyperphosphorylated on Tyr634, Tyr702, and Tyr703 in lung and liver metastasis-derived cell lines [99]. In addition, pancreatic ductal adenocarcinoma cell lines with shRNA knockdown of Axl resulted in reduced tumor growth and metastasis in vivo [66]. Overall, these studies revealed that Axl had potential as a therapeutic target in pancreatic cancer.

7. Axl as a therapeutic target

7.1 Antibodies

Various mAbs specific for Axl have been developed to block Axl activity or as delivery vehicles for radioisotopes or toxins. An early study by the Pomper group [100] demonstrated that radiolabeled antihuman Axl mAbs can be effective for imaging pancreatic cancer and lung cancer xenografts, thus providing a potential new strategy to identify Axl-positive tumors for staging and therapeutic monitoring. Axl mAbs that are neutralizing and inhibit Gas6-mediated activation of Axl have been investigated in multiple preclinical models of cancers. For example, 20G7-D9, a neutralizing anti-Axl mAb, was found to inhibit cell migration, invasion in vitro, and block the growth and metastasis of Axl-positive triple-negative breast cancer (TNBC) xenografts in vivo [101]. Another Axl mAb, YW327.6S2, enhanced the therapeutic efficacy of VEGF and EGFR inhibitors as well as chemotherapy in NSCLC and breast cancer models by inducing tumor cell apoptosis, reducing tumor-associated vascular density, and inhibiting the secretion of inflammatory cytokines and chemokines from tumor-associated macrophages [102]. Another mAb, 10C9, developed by the Lorens laboratory and BerGenBio, had modest effects on controlling primary pancreatic xenografts but potently inhibited metastasis, similar to the effect of systemic warfarin therapy [66]. Other Axl mAbs do not block Gas6 binding but reduce Axl expression. For example, antihuman Axl mAbs generated by the Robert group [98] induced Axl internalization and reduced Axl expression, and inhibited the phosphorylation of Akt but did not inhibit Gas6 binding. Furthermore, these antibodies induced Axl targeted antibody-dependent cellular cytotoxicity in vitro, and reduced pancreatic cancer xenograft growth in

vivo [98]. Additionally, neutralizing mAbs that are specific for Gas6 have also been developed and shown to be effective as single agents or in combination with chemotherapy in reducing the growth of pancreatic tumor xenografts [103].

7.2 Decoy receptors and aptamers

Other biologics have been developed to inhibit ligand-induced Axl activation, most notably soluble decoy receptor constructs which capture Gas6 and prevent it from binding Axl. For example, MYD1–72, a decoy receptor construct from the Giaccia group [104] has higher affinity for Gas6 than native Axl and shows strong antitumor activity in breast cancer models. They also found that MYD1–72 enhanced the activity of gemcitabine in orthotopic pancreatic and ovarian cancer models [104]. Short nucleic acid aptamers that have high affinity for Axl have also been developed. These highly selective Axl targeting moieties inhibit Axl kinase activity, presumably by disrupting Gas6-induced Axl activation, and have been shown to reduce lung tumor xenograft growth in vivo [105].

7.3 Small-molecule inhibitors

Small-molecule inhibitors of Axl have been developed by multiple companies and many have entered clinical testing in cancer patients. In fact, some FDA-approved compounds have pharmacologically relevant Axl inhibitory activity. For example, cabozantinib, crizotinib, and foretinib, which were initially developed as inhibitors for other targets such as Met or VEGFR2, have been shown to achieve their therapeutic effect in part through Axl inhibition [106, 107]. Of the Axl-targeted compounds in development, many inhibit multiple kinases besides Axl. For example, ONO-7475, which prolongs survival of mice bearing acute myeloid leukemia (AML) xenografts, mainly inhibits Axl and MerTK [108]. Other novel multi-RTK inhibitors that showed great preclinical efficacy include RXDX-106 [109], MGCD265/516 [110], and S49076 [111, 112]. Each of these compounds inhibits Axl at a pharmacologically relevant concentration yet they are considered spectrum-selective inhibitors that likely have efficacy due to the inhibition of multiple kinase pathways.

Although the multitarget inhibitors have potent therapeutic efficacy in preclinical models, there is concern that the lack of specificity could result in clinically relevant toxicity. This is especially relevant for inhibitors of all the TAM family members at the same time. Genetic studies using Axl/MerTK double KO or TAM triple KO mice demonstrate that these animals develop systemic autoimmune diseases [113]. Inhibitors that are specific for Axl might be a better choice particularly when Axl inhibition is combined with other therapies to overcome therapy resistance. The most advanced selective Axl inhibitor is bemcentinib (BGB324). The effect of BGB324 has been investigated in preclinical models of late-stage pancreatic cancer [69]. The authors found that BGB324 significantly enhanced gemcitabine efficacy in multiple models (the models included genetically engineered mouse models, syngenic implants, and patient-derived xenografts [PDXs]). BGB324 suppressed proliferation, induced apoptosis, drove epithelial differentiation, and increased the expression of nucleoside transporters associated with tumor uptake of gemcitabine. Additionally, innate immune suppression was reduced by BGB324 as evidenced by decreased MDSCs, M2-type macrophages, and TBK1/NF- κ B signaling. BGB324 has been investigated by multiple other groups, where it showed antitumor and antimetastatic effects and enhanced chemotherapy

and targeted therapy in preclinical models of pancreatic cancer, breast cancer, glioblastoma, prostate cancer, chronic myeloid leukemia, ovarian cancer, and uterine serous cancer [16, 79, 114, 115, 116, 117, 118]. Another novel selective Axl inhibitor is TP-0903. In preclinical models, TP-0903 sensitized preclinical lung cancers to erlotinib and diminished FLT3 inhibitor resistance of AML cells [119, 120].

An alternative strategy to inhibit Axl is to consider the use of low-dose warfarin, a vitamin K antagonist that prevents γ -carboxylation of Gas6, thereby inhibiting ligand-induced activation of Axl. This strategy has been validated in preclinical models of pancreatic cancer [66].

7.4 Axl-related immune therapy

Recently, Axl has been exploited as a target for immune therapy. For example, anti-Axl chimeric antigen receptor (CAR)-T-cell therapy was used to kill Axl-positive myelogenous leukemia cells and TNBC cells with promising efficacy [121, 122]. Axl has also been investigated as a target for antibody drug conjugates (ADCs). Preclinical studies validated the efficacy of the approach in mice bearing melanoma, lung, pancreatic, and cervical cancer PDXs [123].

8. Conclusion

Axl is an attractive therapeutic target based on its function in pancreatic cancer. It promotes metastasis, decreases the efficacy of standard therapy and contributes to the immunosuppressive microenvironment of pancreatic tumors. Targeting Axl is also feasible, since multiple strategies have been developed and confirmed to have good therapeutic benefit in preclinical models of pancreatic cancer. These studies provide a solid rationale for exploring Axl-targeted therapies in clinical trials.

9. Expert opinion

Although the median 5-year survival for patients diagnosed with pancreatic cancer is slowly increasing, this disease remains a lethal cancer and is predicted to be the number two overall cause of cancer-related deaths within the next decade [124]. Metastasis and therapy resistance are two major roadblocks for therapeutic efficacy in pancreatic cancer patients. Over 80% of the patients are diagnosed at a stage when the tumor has already locally advanced or metastasized. Additionally, pancreatic cancer is innately resistant to or rapidly acquires resistance to standard chemotherapy and current immune oncology approaches. Strategies that enhance the efficacy of standard of care and/or immune therapy are likely the most efficient route to improve overall survival in this disease.

The inhibition of Axl is an attractive target for improving the outcome of pancreatic cancer patients. Axl is expressed in a high percentage of human pancreatic cancers, where it is associated with worse outcome. Axl can promote tumor invasion and metastasis and is a critical component of resistance to chemotherapy, targeted therapy, and immune therapy. There are multiple tractable strategies to inhibit Axl, many of which are being tested in cancer patients. While Axl inhibition in pancreatic cancer patients has not been initiated,

there are multiple trials in the planning phase to test various Axl inhibitors in these patients. There is considerable enthusiasm for Axl inhibition as a therapeutic strategy, yet several aspects of Axl biology in pancreatic cancer require additional investigation.

First, it is clear that Axl can be expressed by tumor cells, fibroblasts, and immune cells in the pancreatic tumor microenvironment. Preclinical studies strongly support that Axl on tumor cells is critical for pancreatic cancer progression in Axl-expressing tumors [66]. However, the contribution of Axl on stromal cells to pancreatic tumor growth and spread is not clear. There is preclinical evidence that systemic therapy with BGB324 can result in changes to innate immune cells in pancreatic tumors [69] but whether this effect is due to the direct inhibition of Axl on immune cells or a change in tumor cell cytokine profile is not known. Additionally, Axl activity can repress NK cell antitumor function but the contribution of NK cell activity to the antitumor effect of drugs such as BGB324 or Axl-targeted biologics has not been investigated. These questions are particularly relevant for immune therapy in pancreatic cancer. Immune checkpoint blockade has thus far been ineffective in pancreatic cancer and it is feasible that the interruption of Axl signaling in the pancreatic tumor microenvironment might facilitate immune therapy efficacy by altering the immune landscape of pancreatic cancers to favor innate and adaptive immune responses.

Another challenge that should be addressed is the function of Axl in tumor cell heterogeneity. The Robert group demonstrated that the strongest expression of Axl was in invasive tumor cells, frequently of anaplastic morphology, localized at the periphery of tumors [98]. This suggests that Axl is not universally expressed in all pancreatic cancer cells in a given lesion. The contribution of Axl-positive cancer cells to tumor progression needs to be defined with rigor. Are Axl-positive cells those that have already entered a plastic state? Did they enter that plastic state due to the inherent stress of the microenvironment? Is this a reversible phenotype? In the MMTV-PyMT model, the Axl-positive, mesenchymal state metastasis-initiating cells had a higher capacity to survive circulation and extravasate. After arriving at the distant metastatic site, the same population of cells reverted into Axl-negative cells with decreased motility and increased proliferation, facilitating colonization [67]. In melanomas, Axl-positive cancer cells are rare before treatment. BRAF and MEK inhibitors enriched an Axl-positive, drug-resistant subpopulation, and induced Axl expression in Axl-low cells to gain resistance [123]. Based on these results, the Axl-positive subpopulation in pancreatic cancer is a high-value target population. This approach may also provide improved understanding of how Axl functions in tumor cell plasticity. Highlighting the importance of Axl-positive pancreatic cancer cells also provides a strong rationale for Axl as a therapeutic target.

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List of abbreviations used:

ADC	antibody drug conjugate
AML	acute myeloid leukemia
AP-1	activator protein 1
CAR	chimeric antigen receptor
Cbl-b	casitas B-lineage lymphoma proto-oncogene-b
CTC	circulating tumor cell
DC	dendritic cell
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EMT	epithelial-to-mesenchymal transition
ERK1/2	extracellular signal-regulated kinase 1/2
FLT3	fibromyalgia syndrome-like tyrosine kinase 3
FNIII	fibronectin type III
Gas6	growth arrest-specific gene 6
Gla	γ -carboxyglutamate
Grb2	growth factor receptor-bound protein 2
HDPE	human ductal pancreatic epithelial
HR	homologous recombination
HSC	hepatic stellate cell
IFN	interferon
Ig	immunoglobulin
IL-15	interleukin-15
KO	knockout
LG	laminin G
mAb	monoclonal antibody
MMP	matrix metalloproteinase
MZF1	myeloid zinc finger gene-1
NK	natural killer

NSCLC	non-small-cell lung cancer
PDX	patient-derived xenografts
PI3K	phosphatidylinositol-3-kinases
PKC	protein kinase C
PLC	phospholipase C
PROS	protein S
PS	phosphatidylserine
ROS	reactive oxygen species
RTK	receptor tyrosine kinase
Sp	specificity protein
TAM	Tyro3, Axl, and MerTK
TLR	toll-like receptor
TNBC	triple-negative breast cancer
Treg	regulatory T cell
VSMC	vascular smooth muscle cell
WT	wild-type

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Article highlights

- High Axl expression and activity are associated with poor prognosis, metastasis, and chemoresistance in multiple types of cancers, including pancreatic cancer.
- Pharmacologic small-molecule inhibition of Axl in preclinical studies inhibits tumor growth and metastasis, prolongs animal survival, attenuates immunosuppression, and sensitizes tumors to standard therapies.
- Novel strategies to inhibit Axl involve using chimeric antigen receptor T cells, antibody drug conjugates, and soluble decoy receptors.

Figure 1: Canonical activation of Axl.

The receptor tyrosine kinase Axl consists of two immunoglobulin (Ig) domains, two fibronectin type III (FNIII) domains, one single-pass transmembrane domain, and the tyrosine kinase domain. Its ligand Gas6 is a 75 kDa protein with a γ -carboxyglutamate [17] domain, four epidermal growth factor (EGF)-like repeats, and two laminin G (LG)-like domains. Once the LG domain of Gas6 binds to the Ig domain of Axl, it induces the dimerization of the Gas6/Axl binding complex. Ligand-induced activation of Axl occurs only after the Gla domain of Gas6 binds to phosphatidylserine (PS), which is on the outside of tumor cells, apoptotic cells, viruses, and so on. Upon Gas6 stimulation, several tyrosine residues of Axl get autophosphorylated and serve as docking sites for multiple substrates, including the P85 subunit of phosphatidylinositol-3-kinases (PI3K), growth factor receptor-bound protein 2 (Grb2), phospholipase C (PLC), c-Src, Elmo, and 14-3-3- ζ .

Figure 2: Function of Axl in cancer.

A) Axl expression can be elevated by TGFβ and epithelial-to-mesenchymal (EMT) transcription factors. Axl in turn regulates the expression of EMT markers through the PI3K/Akt pathway. Gas6-stimulated Axl activation can also induce EMT transcription factors. Axl-induced Elmo activation can increase the expression of Rac. Finally, Axl activation can enhance the expression of matrix metalloproteinases (MMPs). **B)** Axl can be upregulated in cancer cells after therapy and help cancer cells gain resistance through the regulation of homologous recombination (HR)-mediated DNA repair, cell plasticity, epidermal growth factor receptor (EGFR) nuclear translocation, and CDC2 dephosphorylation. **C)** Axl on tumor cells can regulate PD-L1 expression through the PI3K/Akt pathway and MHC1 expression through STAT1. Axl activation on tumor cells and immune cells, such as NK cells, also leads to an immunosuppressive cytokine and chemokines profile, which results in increased M2 macrophages, and decreased T cells and DCs in the tumor microenvironment.

Table 1:

Clinical trials of therapies targeting Axl

Compound	Target	IC50 for Axl	Phase	Disease	Treatment	Clinical trial	Sponsor
Cabozantinib	Axl, MET, VEGFR, KIT, FLT3	7nM	II	NSCLC	Cabozantinib	NCT01639508	Memorial Sloan Kettering Cancer Center
			I and II	Urothelial cancer	Cabozantinib + Niraparib	NCT03425201	Fundacion CRIS de Investigacion para Veneer el Cancer
Crizotinib	Axl, ALK, MET, RON, ROS1	294nM	II	Hematologic cancers, solid tumors, metastatic cancer	Crizotinib	NCT02034981	UNICANCER
ONO-7475	Axl/MerTK	0.7nM	I	Acute leukemia	ONO-7475	NCT03176277	Ono Pharmaceutical Co. Ltd
RXDX-106	TYR03, Axl, MerTK, c-MET	3.4nM	I	Advanced or metastatic solid tumors	RXDX-106	NCT03454243	Ignitya, Inc.
BPL-9016M	C-MET/Axl	9nM	I	Solid tumors	BPL-9016M	NCT02478866	Beta Pharmaceuticals Co., Ltd.
MGCD265	Axl, MerTK, MET, VEGFR2, DDR2, PDGFRa/B	5.3nM	I	Advanced cancer	MGCD265	NCT00697632	Mirati Therapeutics Inc.
MGCD516	Axl, MerTK, MET, VEGFR, FLT3, KIT, PDGFRa, DDR1-2	1.5nM	I/II	Advanced cancer	MGCD516	NCT02219711	Mirati Therapeutics Inc.
INCB081776	Axl/MerTK	0.61nM	I	Advanced solid tumors	INCB081776 ± Nivolumab	NCT03522142	Incyte Corporation
Bemcentinib (BGB324)	Axl, Tie-2, FLT4	14nM	I and II	Non-small cell lung cancer	Bemcentinib + Erlotinib	NCT02424617	BerGenBio ASA
			I and II	Acute myeloid leukemia, myelodysplastic syndromes	Bemcentinib ± Cytarabine or Decitabine	NCT02488408	BerGenBio ASA
			II	Lung cancer metastatic, NSCLC stage IV, Adenocarcinoma of lung	Bemcentinib + Pembrolizumab	NCT03184571	BerGenBio ASA
			II	Triple negative breast cancer, inflammatory breast cancer stage IV	Bemcentinib + Pembrolizumab	NCT03184558	BerGenBio ASA
TP-0903	Axl, MerTK, Tyro3, Aurora A/ B, JAK2, ALK, ABL1, CHEK1	27nM	I	Advanced Solid Tumors, EGFR Positive NSCLC, Colorectal Carcinoma,	TP-0903	NCT02729298	Tolero Pharmaceuticals, Inc.

Compound	Target	IC50 for Axl	Phase	Disease	Treatment	Clinical trial	Sponsor
				Recurrent Ovarian Carcinoma, BRAF-Mutated Melanoma			
Warfarin	Gas6		I	Pancreatic cancer	Warfarin	NCT03536208	UT Southwestern Medical Center
CCT301-38 CAR-T	Autologous T Cell Modified CAR for Axl		I	Renal cell carcinoma	CCT301-38	NCT03393936	Shanghai Sinobioway Sumterra Biotech
HuMax-AXL-ADC	AXL-specific antibody-drug conjugate		I and II	Ovarian cancer, cervical cancer, endometrial cancer, NSCLC, thyroid cancer, melanoma, sarcoma	HuMax-AXL-ADC	NCT02988817	Genmab
CAB-AXL-ADC	AXL-specific antibody-drug conjugate		I and II	Solid Tumor, NSCLC, castration-resistant prostate cancer, pancreatic cancer	CAB-AXL-ADC	NCT03425279	BioAtla, LLC