Axl Kinase as a Key Target for Oncology: Focus on Small Molecule Inhibitors

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Abstract

Receptor tyrosine kinases (RTK) are transmembrane receptors that regulate signal transduction in cells. As a member of the TAM (Tyro-3, Axl, Mer) RTK subfamily, Axl regulates key processes such as cell growth, migration, aggregation, and apoptosis through several pathways. Its overexpression/overactivation has been underlined in several conditions, especially cancers, and in both chemotherapy and targeted therapy sensitivity loss. In this review, we propose to highlight the therapeutic implication of Axl, starting with the pathways it regulates, validating its interest as a therapeutic target, and defining the tools available to develop strategies for its inhibition. We especially focus on small molecule inhibitors, their structure, inhibition profile, and development stages. Mol Cancer Ther; 13(9); 2141–8. ©2014 AACR.

Introduction

TAM family and Axl

Axl (Ark, Ufo) is an oncoprotein originally isolated from 2 patients with chronic myeloid leukemia (CML) and a chronic myeloproliferative disorder in 1988 (1–3). Axl was identified as a member of the TAM family, a receptor tyrosine kinase (RTK) subfamily comprising Tyro-3 (also called Sky), Axl and Mer. Axl is an ubiquitous receptor predominantly expressed in the brain (hippocampus and cerebellum), in immunity cells, blood platelets, endothelial cells, skeletal muscle, testis, heart, liver, and kidney (4). TAM receptors were initially found in cancer cells (2, 4), which guided the research in the field of oncology. These RTKs contribute to a carcinogenesis phenomenon via overexpression or ectopia, regulating processes such as migration, cell survival, and proliferation (3, 5). They are involved in several cancers, such as pancreas, breast, prostate, non–small cell lung cancer (NSCLC), CML, and other cancers (6).

Structurally, TAM receptors possess an extracellular, a transmembrane, and an intracellular domain. The extracellular region is composed of 2 immunoglobulin-like and 2 fibronectin domains that are involved in the binding of the natural ligand (7). In 2005, the crystal structure of this ligand complexed to its receptor Axl (only the extracellular part) was solved (PDB ID code 2C5D), proving that the receptor dimerization occurs without any receptor/receptor (on the extracellular domain) or ligand/ligand contacts (9).

Although classical activation of Axl starts by the binding of its natural ligand Gas6 onto its extracellular domain, several studies have also demonstrated that Axl could be activated by a ligand-independent mechanism or by a cross-talk with other signaling pathways (10). The Gas6–Axl binding induces the dimerization of the RTK, allowing a transautophosphorylation of the cytoplasmic domains. Three tyrosine residues (Y779, Y821, and Y866) are supposed to be phosphorylation sites (11), mostly because they can modulate the interaction of Axl with signaling molecules such as phospholipase C, PI3K, Src, Lck, and Grb2 (9, 11). An analogy with the finding on Mer would underline the possibility of 3 other phosphorylation sites that are conserved among the TAM family (Y666, Y702, and Y763) (refs. 8, 12). However, it is interesting to note that Y866 of Axl is also suspected to be an inhibitor site of phosphorylation (13).

Once activated, the catalytic capacity of Axl increases, enabling other cytoplasmic signaling substrates to be phosphorylated. Depending on the cell type and the environment, the activation of Axl can stimulate various cellular functions (Fig. 1). Through the Src/MAPK/ERK pathway, Axl regulates cell survival and proliferation (14, 15). Through the PI3K/Akt pathway, it regulates survival and stimulates expression of antiapoptotic proteins, notably through NF-kB modulation (16, 17). Through the PI3K/p38/MAPK pathway, Axl regulates cell migration and development stages.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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and proliferation. Activation of Axl, mainly by way of atypical activation, also leads to cell aggregation (18) and a proangiogenic behavior (10, 19). Taken together, these data suggest that Axl is an important regulator of cell survival, proliferation, aggregation, migration, adhesion and is necessary for angiogenesis.

Mechanisms of negative control have been proposed, such as dephosphorylation by the C1-TEN phosphatase, which can bind Axl and whose overexpression correlates with a downregulation of survival, proliferation, and migration (20, 21). Ubiquitination of Axl by ubiquitin ligase C, which leads to its downregulation, has also been studied. Among atypical activation mechanisms, Kamata and colleagues reported that behavior of numerous kinases and phosphatases was modified depending on the intracellular oxidation rate (22). In particular, reactive oxygen species (ROS) generation in cells by hydrogen peroxide adjunction resulted in Axl activation within a short time (23). More importantly, it was shown that this activation did not induce ubiquitination of Axl by ubiquitin ligase C, as it is the case of classical Gas6 activation. As a matter of fact, oxidative stress might inhibit Axl ubiquitination and therefore downregulation through lysosome degradation (24).

Involvement in cancers

Knowing the pathways regulated by Axl, altered activity of this RTK has been detected in a variety of cancer types, such as pancreas, breast, prostate, NSCLC, CML, and other cancers (25). To date, no activating mutations in the Axl gene have been implicated in malignant transformations.

The Axl kinase is postulated to contribute to a carcinogenesis phenomenon via overexpression or ectopia. In particular, Axl overexpression has been reported in various oncology indications, and the expression level of Axl is directly proportional to tumor grade and predicts poor patient survival rate. In addition, Axl is involved in metastasis as an essential regulator of the epithelial–mesenchymal transition (EMT) process in cancer cells (26).
Since approximately the 2000s, several studies have demonstrated a strong and positive impact of inhibiting Axl in cancer. The inhibition of Axl led to the decrease of proliferation and survival in pancreatic cancer cell lines (27). In breast cancer, its inhibition decreased the migration and invasion of cancer cells (28). In prostate cancer, migration, invasion, and proliferation were decreased by the inhibition of Axl (29). In NSCLC, silencing of Axl inhibited tumor growth (30).

Axl is a factor of poor prognosis when implicated in cancer development (27, 31). Therefore, its overexpression is related to high level of recurrence (32) as well as resistance mechanisms toward the effects of other anticancer drugs, such as imatinib (Glivec; ref. 33), erlotinib (Tarceva; refs. 34, 35), and lapatinib (Tykerb; ref. 36), on several cancers [leukemia, gastrointestinal stromal tumors (GIST), breast, and lung cancers].

Taken together, all these data validate Axl as a therapeutic target to treat several cancers, and many research scientists are developing strategies to inhibit this RTK. Different strategies have been elaborated, like inhibiting the binding of Axl to its activator ligand, competing the ATP to lower its activity, or reducing the expression of the protein itself.

Biophysical and Statistical Data

So far, one X-ray tridimensional structure is available for Axl (PDB ID code 2C5D). It concerns the extracellular part binding its ligand Gas6, showing a ligand–receptor 2:2 assembly (9). To date, there are no published data available about the three-dimensional structure of the kinase domain of Axl.

Kinases with a high identity score toward Axl, such as same family kinases, could be expected to express an inhibition profile similar to Axl. The availability of the crystal structure of Mer (37), Met (38), and IGFIR (39), which are closely related to Axl regarding both the ATP-binding site and the whole kinase domain, allowed Mollard and colleagues (40) to build a predictive homology model of Axl.

In 2011, Metz and colleagues published a comprehensive kinase interaction network. More than 150,000 inhibition data of 3,800 ligands toward 172 kinases were collected (41). To determine whether 2 kinases can be classified as related or not, mathematical tools were used. Scaled Shannon entropy (SSE; ref. 42) was used to measure the reliability of information, using the number of data and their dispersion (Supplementary Table S1). The authors determined an SSE of ≥0.4 as minimum threshold. Other tools were used, such as $P_{ij}$ (Hopkins pharmacology interaction strength; ref. 43), $R_{ij}$ (Pearson correlation coefficient), and $T_{ij}$ (activity profile Tanimoto; ref. 44), to assess the relevance of the interaction, using the number of ligands with similar bioactivity on 2 kinases of the ligands tested on both these kinases, and the linear correlation between the variables. The authors determined values on these indexes to establish a strong pharmacologic relationship between 2 kinases as $P_{ij} \geq 0.6$, $R_{ij} \geq 0.45$, and $T_{ij} \geq 0.55$.

According to the authors, more than 90% of kinases that share sequence identity higher than 60% also have $P_{ij} \geq 0.6$. Regarding Axl, only 3 other kinases match these parameters and can therefore be classified as related to Axl: Flt4, MAPK5, and Ret (Supplementary Table S1). Furthermore, Tyro-3, which is the only kinase among the 172 reported on the study that share more than 60% sequence identity with Axl, has a $P_{ij}$ of 0.18. The behavior of Axl is different from the majority of kinases at least from that point of view. None of the kinases used to build the predictive model match more than one criterion except the SSE. (Note that no data were collected on Mer on that article.) Available data on Axl are not only rare but also quite contradictory.

Small Molecule Inhibitors

To our knowledge, no inhibitor clearly designed to inhibit Axl is currently marketed. In particular, none of the listed molecules in the marketed drugs and clinical phase II sections have been developed as preclinical or clinical Axl inhibitors. Most of the anti-Axl developed drug candidates are in preclinical stages. However, some FDA-approved or clinical molecules, developed to inhibit other kinases, were evaluated against Axl given its interest. Some potent Axl kinase inhibitors in development are described in the literature (45, 46). However, as few data are available regarding their selectivity and their application as treatment, we do not describe them here. Here, we summarize properties of molecules that are FDA approved, in clinical trial, or planned to be within the next year. All the molecules listed below are shown in Supplementary Fig. S1.

Marketed drugs

Bosutinib (SKI606, PFS208763, Bosulif; Pfizer) was authorized by the FDA and the European Medicines Agency (EMA) in 2012 for the treatment of patients with Ph $^+$ CML (Philadelphia chromosome-positive CML; refs. 47, 48). It is an inhibitor that mainly targets Bcr-Abl1 and Src (IC$_{50}$ = 1 and 3.5 nmol/L, respectively) and, to a lesser extent, Axl (IC$_{50}$ = 174 nmol/L; refs. 49, 50). Bosutinib is currently in a phase II clinical trial for glioblastomas and breast cancer.

Cabozantinib (XL184, Cometriq; Exelixis) was authorized by the FDA and the EMA in 2012 for the treatment of medullary thyroid cancer (51, 52). It is a multitargeted kinase inhibitor (MTKI) acting on VEGFR-2 (IC$_{50}$ = 0.035 nmol/L), Met (IC$_{50}$ = 1.3 nmol/L), and Axl (IC$_{50}$ = 5.2 nmol/L) as primary targets, as well as on Kit (IC$_{50}$ = 14.3 nmol/L), Fli-3 (IC$_{50}$ = 11.3 nmol/L), and Flt3 (IC$_{50}$ = 7 nmol/L; ref. 53). It is currently in a phase III clinical trial for hepatocellular, renal, and prostate cancers.

Sunitinib (SU11248, Sutent; Pfizer) was authorized by the FDA in 2006 commonly for both indications, renal cell carcinomas and imatinib-resistant gastrointestinal stromal tumors, and in 2011 for pancreatic neuroendocrine tumors. It is an inhibitor targeting Flt3 (IC$_{50}$ = 0.5 nmol/L), VEGFR-2 (IC$_{50}$ = 20 nmol/L), Kit (IC$_{50}$ = 22 nmol/L), and PDGFR (IC$_{50}$ = 4 nmol/L).
Clinical phase II

Foretinib (XL880, GSK1363089; Exelixis, GlaxoSmithKline) is an MTKI targeting mainly Met, VEGFR-2, Ron, Tie-2, and Kit (IC50 = 0.4, 0.86, 3, 1.1, and 3.7 nmol/L, respectively). It also exhibits a significant activity toward Axl (IC50 = 11 nmol/L; refs. 36, 55). It is a type II inhibitor, as illustrated by the X-ray crystal structure of foretinib within Met active site (PDB ID code 3LQ8). It is presently in a phase II clinical trial for NSCLC and in phase I/II for breast cancers.

MGCD265 (Mirati Therapeutics) is a potent inhibitor of Met (IC50 = 1 nmol/L), Ron (IC50 = 2 nmol/L), VEGFR 1/2/3 (IC50 = 3, 3, and 4 nmol/L, respectively), Tie-2 (IC50 = 7 nmol/L), and Axl (1 nmol/L ≤ IC50 ≤ 10 nmol/L; ref. 56). It is currently in phase I/II clinical trials for NSCLC and in phase I for advanced malignancies.

Clinical phase I

BMS777607 (ASLAN002; Aslan Pharmaceuticals and Bristol-Myers Squibb) is a type II (57) Met inhibitor (PDB ID code 3F82; IC50 = 3.9 nmol/L) that also acts on Ron (IC50 = 1.8 nmol/L), Flt-3 (IC50 = 16 nmol/L), and the TAM family Tyro-3 (IC50 = 4.3 nmol/L), Mer (IC50 = 14 nmol/L), and Axl (IC50 = 1.1 nmol/L; ref. 58). It is currently in phase I clinical trial for advanced or metastatic solid tumors.

LY281653 (Eli Lilly Company) is an inhibitor that exhibits strong activity toward Ron (IC50 = 0.8 nmol/L), Met (IC50 = 4.7 nmol/L), Axl (IC50 = 11 nmol/L), and Flt-3 (IC50 = 31 nmol/L; ref. 59). It is currently in a phase I clinical trial for advanced cancers.

S49076 (Servier) is described as an MTKI exhibiting activity toward Met (IC50 = 2 nmol/L), FGFR-1 (IC50 = 68 nmol/L), FGFR-2 (IC50 = 95 nmol/L), FGFR-3 (IC50 = 200 nmol/L), and Axl (IC50 = 56 nmol/L). Its selectivity was evaluated against 442 kinases, among which more than 25 were identified as hits at 100 nmol/L (60). It is currently in a phase I clinical trial for advanced solid tumors.

BGB324 (R428; Rigel Pharmaceuticals, BergenBio) is currently one of the most promising molecules to target Axl regarding both its activity (IC50 = 14 nmol/L) and its selectivity. Indeed, the inhibition of Axl by this molecule was 50 to 100 times lower than for the 133 kinases tested, with the exception of Tie-2 (3 times), Flt-4 (5.5 times), Flt-1 (8 times), Ret (9 times), Abl (9.3 times), and the other members of TAM family Tyro-3 (14 times) and Mer (16 times; ref. 61). BGB324 just entered clinical phase I in 2013 for aggressive and metastatic cancers.

Preclinical stage

TP0903 (Tolero Pharmaceuticals) exhibits strong activity toward Axl (IC50 = 27 nmol/L; ref. 40). It was screened against a panel of 40 kinases at 200 nmol/L and exhibited more than 80% inhibition on 11 kinases, including Aurora A, Jak2, Alk, and Abl, with higher inhibition percentages than Axl, as well as the rest of the TAM family. It is currently in preclinical stages for pancreatic cancer and glioblastomas (62).

SGI7079 (Tolero Pharmaceuticals) is an Axl inhibitor (IC50 ≤ 30 nmol/L) that also exhibits a strong activity toward other kinases, such as Flt-3, Mer, TrkA and B, Ret, Yes, Jak2, VEGFR-2, JNK3, and Abl at the same range (IC50 ≤ 30 nmol/L), as well as 12 others with IC50 below 100 nmol/L (63). It is supposed to enter clinical trial phase I in 2014 for NSCLC and other tumor type indications.

Suspended studies

Amuvatinib (SGI-0470-02, MP470; SuperGen, Inc., now known as Astex Pharmaceuticals, Inc.) is an MTKI targeting c-Kit, PDGFRα, Met, Flt-3 (IC50 = 10, 40, and 81 nmol/L, respectively). It was shown to inhibit the phosphorylation of Axl in MDA-MB231 breast cancer cells (EC50 = 1 µmol/L). It was investigated in a phase II clinical trial for small cell lung cancer, but its clinical development was discontinued in 2012 (64).

SNS314 (Sunesis Pharmaceuticals) is a pan-Aurora kinase inhibitor. It exhibits strong activity toward its primary targets Aurora A/B/C (IC50 = 9, 31, and 3.4 nmol/L, respectively), as well as toward 7 of 219 kinases (within 100-fold), including Axl (IC50 = 84 nmol/L; ref. 65). It was investigated in a phase I clinical trial for solid tumors but suspended in 2010 as a maximum tolerated dose was not established in the trial, and no responses were observed (66).

Medicinal Chemistry

The Axl inhibitors discussed above can be classified into 2 groups according to their chemical structures.

The first scheme (Fig. 2A) includes inhibitors bearing a hinge-binding heterocycle bearing a solubilizing group (N-methylpiperazine), linked to a hydrogen bond acceptor–substituted phenyl group by a linker. Three inhibitors of the precedent list obey this description.

As their structure is similar, it is interesting to look at their inhibition profile toward their respected target kinases (Table 1). TP0903 and SGI7079 share a similar inhibition profile on the target kinases of the first scheme (Fig. 2A). They also possess at least 2 hydrogen bond donor/acceptor on their hinge-binding part and a similar hydrophilic part. Bosutinib, however, possesses only one hydrogen bond acceptor on its hinge-binding heterocycle. The major part of its activity is therefore related to affinity for less-conserved parts of the kinases, reducing the array of hit kinases, as Aurora A, for instance.

Although these 3 inhibitors exhibit the same structural skeleton as described in Fig. 2A, heterocycle and substituent nature induce a very specific inhibition profile for each of them on the targeted kinases. It is difficult to establish a relationship between structure and activity of
these kinases; nevertheless, these 3 inhibitors exhibit 3 IC₅₀ lower than 200 nmol/L on the presented kinase panel, with a high inhibition of Axl, especially for TP0903 and SGI7079.

The second scheme that can be observed (Fig. 2B) presents 5 inhibitors with a hinge-binding heterocycle, with or without bearing a solubilizing group, linked to an optionally ortho-fluoro substituted phenyl group by an oxygen linker, followed by a sequence of 2 to 4 hydrogen donors/acceptors linked to an optionally para-fluoro substituted phenyl group. The oxygen linker is in para position of a nitrogen atom of the hinge-binding heterocycle, with the exception of LY2801653.

Because the structure of inhibitors is highly similar, we look at their inhibition profile toward their respected target kinases (Table 1). Cabozantinib and foretinib structurally differ only by their solubilizing group and a fluor substituent. These slight differences lead to significant variation in the inhibition activity of VEGFR and Ron kinases (25-fold). MGCD265 is also structurally similar to foretinib and cabozantinib, with the notable exception of their hinge-binding heterocycle, the presence of a fluorine atom at the other edge of the molecule, and the position of one of its hydrogen bond donor. However, these small differences still confer MGCD265 an inhibition profile very close to that of foretinib. BMS777607 and LY2801653 mainly differ from their hinge-binding scaffold and, therefore, display a similar inhibition profile.

These 5 inhibitors strongly inhibit Met and Axl, with a low nanomolar activity. They exhibit similar kinase inhibition profiles on the targeted kinases with at least 3 IC₅₀ less than 10 nmol/L, including Axl.

Of all the previously cited Axl inhibitors, three inhibitors are missing in the last 2 schemes: sunitinib, S49076, and Sunitinib, S49076.
and BGB324. Their structures are quite different from those of the other inhibitors and from one another and were, therefore, unsorted.

Discussion

Axl has been validated as a therapeutic target of choice for several cancers as well as implicated in many resistance phenomena to accurate therapies. Despite its major interest, so far no Axl-specific inhibitor exists on the market. Moreover, none of the marketed or clinical phase II molecules have been designed to target Axl. Thus, it has to be stated that BGB324, the first-in-class Axl inhibitor currently in clinical phase I, and TP0903, which is in preclinical development, are possibly the most specific Axl inhibitors. The design of such an Axl inhibitor is a challenge due to the absence of three-dimensional structure of its kinase domain. Moreover, a statistical study showed that the inhibition profile of Axl cannot be related to sequence identity as could be done for 90% of all kinases. Several molecules do inhibit Axl, but most of them are MTKIs, and Axl is an additional target, not the main one. Most of these molecules match 2 schemes that can be related to type I inhibitors, targeting the active conformation, called DFG-in, and type II inhibitors known to target the inactive conformation, called DFG-out. Thus, experimental evidence of such thermodynamically stabilized DFG-out conformation has only been reported for a few kinases, excluding Axl (67, 68). These patterns may provide clues leading one to believe the existence of a DFG-out conformation for Axl.

Although it has not been addressed in this review, the expression of Axl is upregulated in a variety of other diseases, including endometriosis and cardiovascular diseases (69, 70). In addition, Axl appears to play a crucial role in innate immunity (71). Small molecule inhibitors of the TAM receptors may be reasonable candidates for potential therapies in acute sepsis and for new generation of vaccine adjuvants for immunization (71). Also, it has to be mentioned that other ways to regulate the activation of Axl are being developed, such as monoclonal antibodies targeting the kinase or its ligand Gas6, as well as aptamers to downregulate its expression (72, 73).

Conclusion

Since the discovery of Axl in 1988, Axl has been studied and validated as a therapeutic target of choice for several diseases. Despite the fact that no specific Axl inhibitor can be found on the market today, there has been increasing interest in Axl, leading to a better comprehension of this kinase moving forward.

Design and development of Axl inhibitors is a challenge due to the absence of three-dimensional structure of its kinase domain, and only classical medicinal chemistry approaches have improved scientific knowledge about Axl. Statistical studies have shown that the inhibition profile of Axl cannot be related to sequence identity as could be done for 90% of kinases. Several molecules do inhibit Axl, but most of them are MTKIs, and Axl is an additional secondary target, not the main one.

There is no doubt that more studies will be carried out, providing the data needed to achieve important findings about structural, chemical, pharmacological, and therapeutic aspects of this target. An Axl-specific inhibitor will be an efficient treatment for some aggressive diseases such as cancers and for counteracting Axl-induced resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Axil Kinase as an Emerging Key Target for Drug Discovery

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Molecular Cancer Therapeutics


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Correction: Axl Kinase as a Key Target for Oncology: Focus on Small Molecule Inhibitors

In this article (Mol Cancer Ther 2014;13:2141–8), published in the September 2014 issue of Molecular Cancer Therapeutics (1), Figure 2 and Supplementary Figure 1 remained from an unrevised version of the manuscript and present incorrect structures for compounds BMS777607, MGCD265, SGI7079 and S49076. The correct structures of these compounds are presented below in Figure 2 and Supplementary Figure 1. The authors apologize for the errors.

**Reference**


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