

REVIEW

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AXL as immune regulator and therapeutic target in Acute Myeloid Leukemia: from current progress to novel strategies

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Abstract

Until recently, treatment options for patients diagnosed with Acute Myeloid Leukemia (AML) were limited and predominantly relied on various combinations, dosages, or schedules of traditional chemotherapeutic agents. Patients with advanced age, relapsed/refractory disease or comorbidities were often left without effective treatment options. Novel advances in the understanding of leukemogenesis at the molecular and genetic levels, alongside recent progress in drug development, have resulted in the emergence of novel therapeutic agents and strategies for AML patients. Among these innovations, the receptor tyrosine kinase AXL has been established as a promising therapeutic target for AML. AXL is a key regulator of several cellular functions, including epithelial-to-mesenchymal transition in tumor cells, immune regulation, apoptosis, angiogenesis and the development of chemoresistance. Clinical studies of AXL inhibitors, as single agents and in combination therapy, have demonstrated promising efficacy in treating AML. Additionally, novel AXL-targeted therapies, such as AXL-specific antibodies or antibody fragments, present potential solutions to overcome the limitations associated with traditional small-molecule AXL inhibitors or multikinase inhibitors. This review provides a comprehensive overview of the structure and biological functions of AXL under normal physiological conditions, including its role in immune regulation. We also summarize AXL's involvement in cancer, with a specific emphasis on its role in the pathogenesis of AML, its contribution to immune evasion and drug resistance. Moreover, we discuss the AXL inhibitors currently undergoing (pre)clinical evaluation for the treatment of AML.

Keywords AXL, Immunoregulation, Therapy, Acute Myeloid Leukemia

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Introduction

Acute Myeloid Leukemia (AML) is one of the most challenging hematological malignancies and is characterized by clonal expansion of myeloid blasts in the bone marrow (BM) often leading to circulating blasts in peripheral blood [1]. The uncontrolled growth of blasts leads to the suppression of normal hematopoiesis, causing BM failure and impairing the production of normal blood cells [2]. AML primarily affects older adults, with a median age at diagnosis around 68 years and a 5-year survival rate below 20% for patients between 60 and 74 years of age [3].

AML is a highly heterogeneous disease with regard to cell morphology, cytogenetics and gene mutations [4]. The prognosis of AML patients is variable and is based on clinical features (e.g., age, comorbidities and general performance) and underlying genetic features, including both cytogenetic and molecular aberrations [5]. Common genetic mutations observed in AML include mutations in genes encoding transcription factors (e.g., *RUNX1*, *CEBPA*), signaling molecules (e.g., *RAS*), epigenetic modifiers (e.g., *DNMT3A*, *IDH1/2*), and tumor suppressors (e.g., *TP53*). Approximately 20% of the AML patients harbor internal tandem duplication (ITD) mutations in the Fms-like tyrosine kinase-3 (*FLT3*) gene, which are consistently associated with a poor prognosis [6, 7]. Another common genetic alteration is the nucleophosmin 1 (*NPM1*) mutation, occurring in approximately one-third of AML cases. The *NPM1* mutation is considered a favorable prognostic marker when detected as an isolated mutation (i.e., without concurrent *FLT3*-ITD mutations) and is associated with higher rates of complete remission (CR) and overall survival (OS) [8].

However, despite significant advances in the understanding of AML pathogenesis, the main treatment for most AML patients remains standard chemotherapy, including cytarabine combined with an anthracycline, and the use of allogeneic hematopoietic stem cell (HSC) transplantation [9]. During the past years, the Food and Drug Administration (FDA) also approved numerous targeted therapies for AML, including *FLT3* inhibitors, isocitrate dehydrogenase 1 (*IDH1*) inhibitors, B-cell lymphoma 2 (*BCL-2*) inhibitors and hypomethylating agents, allowing a more personalized treatment approach [10].

In recent years, the receptor tyrosine kinase (RTK) AXL has been identified as another critical player in AML pathogenesis. AXL was found to be overexpressed in AML patients and was associated with a poor prognosis [11, 12]. Since AXL regulates several processes involved in cancer pathogenesis, including cell survival and proliferation, angiogenesis, epithelial-to-mesenchymal transition (EMT), stem cell maintenance and immunological responses, therapeutic targeting of the AXL signaling pathway became an attractive treatment approach [13].

In this review, we discuss the function of AXL in maintaining normal physiological functions, particularly in immune regulation, and examine its involvement in cancer, with an emphasis on AML. We explore AXL's contribution to AML pathogenesis, including mechanisms of immune evasion and drug resistance, and provide an overview of current (pre)clinical AXL inhibitors being evaluated for AML.

AXL function in physiological conditions and cancer

AXL structure and activation regulation

AXL is a member of the TAM (TYRO3, AXL, MERTK) family of RTKs, which are composed of an extracellular domain (ECD), containing tandem repeats of immunoglobulin (Ig)- and fibronectin type 3 (FN-III)-like domains, a transmembrane domain and an intracellular domain (Fig. 1). The intracellular domain contains a catalytically competent kinase defined by a unique, conserved KWIAIES sequence [14]. All TAM receptors are single-pass transmembrane receptors that bind to extracellular ligands, more specifically growth-arrest specific protein 6 (*GAS6*), protein S, Tubby, Tubby-like protein 1 and Galectin-3 [15–17]. Ligand-receptor interaction triggers receptor dimerization and autophosphorylation [18–20], which serves as a molecular switch, activating downstream signaling pathways that modulate cell survival, proliferation, and immune responses [21–23].

GAS6, a vitamin K-dependent protein, serves as the high-affinity ligand for the AXL receptor [19, 24]. Its name reflects the discovery in growth-arrested cells and refers to its regulating role in cellular homeostasis [25]. The specific interaction between *GAS6* and AXL, facilitated by the unique carboxy-terminal “LG” (loop and ‘G’ γ -carboxyglutamic acid) domains of *GAS6* and the Ig-like domains of AXL, initiates a cascade of downstream signaling pathways such as the phosphatidylinositol 3-kinase/Protein kinase B (PI3K/AKT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. Activation of these pathways orchestrate context-specific cellular responses, ranging from cell survival and proliferation to migration and differentiation [13, 23, 26]. However, evidence points to a constitutive *GAS6*/AXL interaction, which by itself is insufficient to trigger the activation of these downstream effectors [27]. Additionally, *GAS6*-independent mechanisms of AXL activation have been reported [28]. Upon AXL overexpression, excess AXL protein may lead to homophilic binding of ECDs on adjacent cells [29], or even ligand-independent homodimerization [30], with subsequent downstream activation in either case. Other studies reported ligand-independent AXL activation in response to hydrogen peroxide via

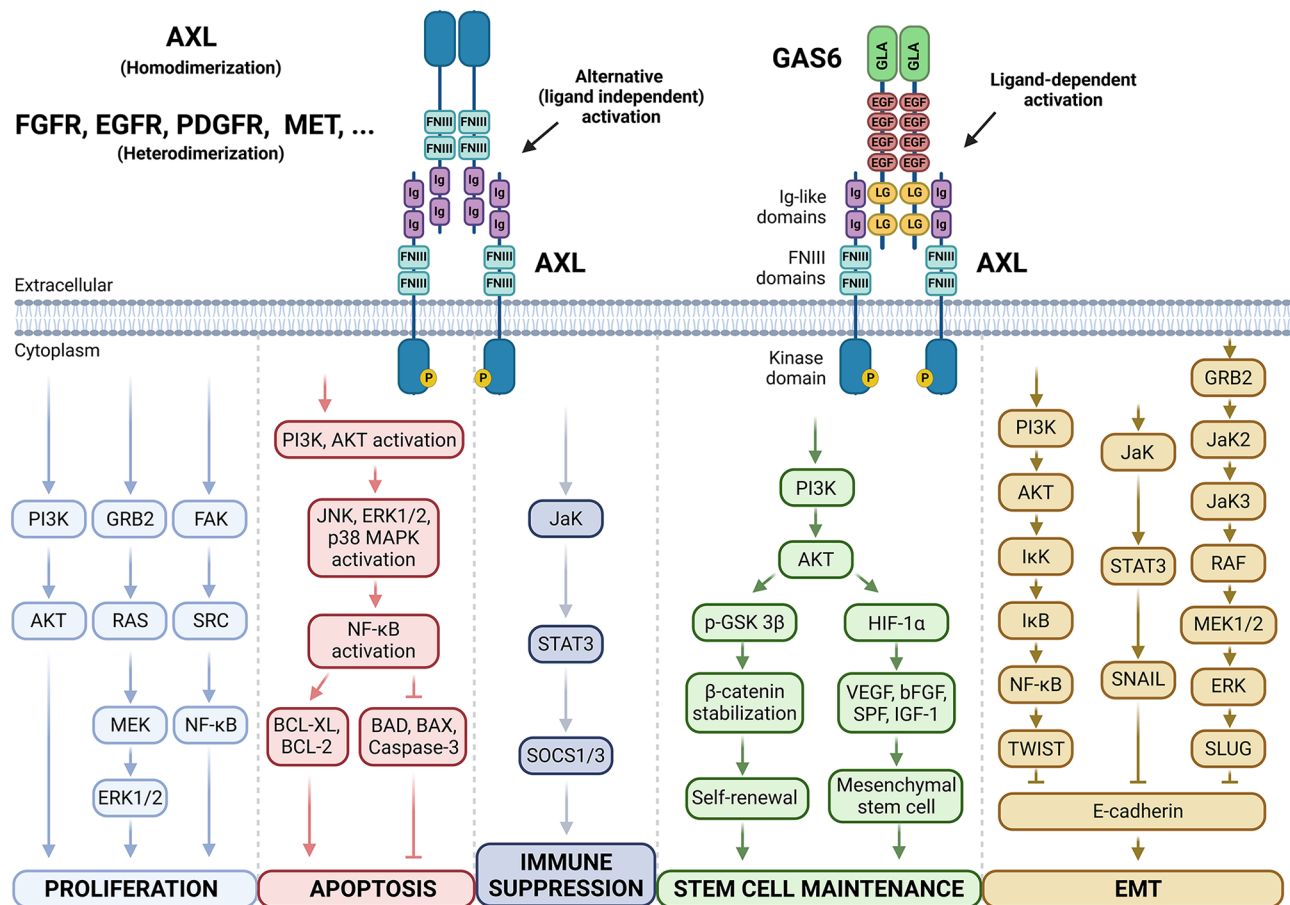


Fig. 1 Schematic representation of the basic structure, activation and downstream signaling pathways of AXL. AXL is composed of two immunoglobulin (Ig)-like repeats and two fibronectin type III (FNIII)-like repeats, a transmembrane domain and an intracellular kinase domain. The latter can be phosphorylated through classical ligand-mediated activation upon interaction with GAS6, a vitamin K-dependent protein that binds AXL with higher affinity compared to TYRO3 or MERTK. The Gla domain of GAS6 allows for cell membrane contact and the LG domains bind the Ig-like domains of AXL. Alternatively, AXL can undergo ligand-independent activation via interaction with another AXL receptor (homodimerization), or via interaction with other receptor tyrosine kinases (heterodimerization). Upon interaction, AXL will undergo dimerization and phosphorylation, which subsequently leads to the activation of a plethora of downstream signaling cascades contributing to, among others, proliferation, apoptosis, immune suppression, stem cell maintenance or epithelial-to-mesenchymal transition (EMT). PI3K, Phosphatidylinositol-3-kinase; GRB2, Growth Factor Receptor-Bound Protein 2; FAK, Focal Adhesion Kinase; AKT, Protein Kinase B; RAS, Rat Sarcoma Virus Oncogene; SRC, Proto-Oncogene Tyrosine-Protein Kinase; MEK, Mitogen-Activated Protein Kinase Kinase; NF-κB, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; ERK1/2, Extracellular Signal-Regulated Kinases 1 and 2; JNK, c-Jun N-terminal Kinase; p38, p38 Mitogen-Activated Protein Kinase; MAPK, Mitogen-Activated Protein Kinase; BAX, BCL-2 Associated X, Apoptosis Regulator; BCL-2, B-Cell Lymphoma 2; JaK, Janus Kinase; STAT3, Signal Transducer and Activator of Transcription 3; SOCS1/3, Suppressor of Cytokine Signaling 1/3; p-GSK 3β, Phosphorylated Glycogen Synthase Kinase 3β; HIF-1α, Hypoxia-Inducible Factor 1α; VEGF, Vascular Endothelial Growth Factor; bFGF, Basic Fibroblast Growth Factor; SPF, Serum Platelet Factor; IGF-1, Insulin-Like Growth Factor 1; IKK, Inhibitor of kappa B Kinase; IKB, Inhibitor of kappa B; TWIST, Twist-Related Protein 1; SNAIL, Snail Family Transcriptional Repressor 1; JAK2/3, Janus Kinase 2/3; RAF, Rapidly Accelerated Fibrosarcoma; SLUG, Snail Family Transcriptional Repressor 2. Figure created with BioRender.com

reactive oxygen species (ROS) [31]. AXL also engages in crosstalk and heterodimerization with both TAM [32, 33] and non-TAM RTKs, such as the fibroblast (FGFR), epidermal (EGFR), platelet-derived (PDGFR) and hepatocyte growth factor receptors (MET), activating downstream pathways regardless the presence of the dimerization partner's ligand (Fig. 1) [34–37]. In this way, AXL can promote resistance to several therapies, including chemotherapy and targeted therapies, or even inhibitors of its partners [38, 39].

The GAS6/AXL axis is subject to complex feedback mechanisms that regulate the intensity and duration of signaling. Negative regulators, such as protein tyrosine phosphatases [40], control AXL phosphorylation levels and prevent excessive activation, while ubiquitin-proteasome-mediated degradation ensures the timely termination of the AXL signaling cascade [41]. In addition, AXL can be cleaved by the proteases a disintegrin and metalloproteinase 10 (ADAM10) and ADAM17 (TACE), resulting in shedding of AXL into the extracellular space. This soluble fragment retains the ability to bind GAS6

through its Ig-like domains and is therefore able to function as a decoy receptor to diminish GAS6 signaling [42]. These regulatory mechanisms contribute to the precision of cellular responses, preventing aberrant signaling and maintaining cellular homeostasis. In addition, the cross-talk between AXL and GAS6 has profound implications on various physiological processes, being a finely tuned system where the balance between receptor activation, ligand availability and the presence of other signaling molecules, can elicit diverse responses tailored to specific physiological requirements [41, 43].

Physiological role of the GAS6/AXL signaling pathway

Under physiological conditions, AXL signaling in endothelial cells, fibroblasts, smooth muscle cells and platelets contributes to angiogenesis, vascular homeostasis, extracellular matrix (ECM) protein production, and cell survival and migration, all playing a role in tissue repair processes.

The function of AXL in immune cells is mainly studied in the context of inflammation. Using HSC-derived natural killer (NK) cells, it has been found that agonistic anti-AXL antibody or recombinant GAS6 specifically upregulated the expression of NK cell-specific receptors (e.g., LY49A, Ly49G2, Ly49C/F/I, NKG2A/C/E) and NK cell-associated molecules (e.g., IL-2R β , perforin, IL-15R α , IFN- γ); suggesting a regulatory role of AXL in NK cell development, activation and effector function [44]. Under physiological conditions, AXL on phagocytic cells, including macrophages, plays a central role in apoptotic cell clearance by binding the “eat-me” signal phosphatidylserine (PS) and triggering PS-mediated efferocytosis during inflammation, which is essential for tissue development and repair. Inflammatory stimuli such as interferon (IFN) and the toll-like receptor (TLR) 3 ligand poly(I: C), upregulate AXL expression in both murine and human macrophages, enhancing the binding of AXL to GAS6, and increasing macrophage ability to engulf and clear apoptotic cells [27, 45].

In dendritic cells (DCs), AXL expression can be upregulated by inflammatory stimuli such as lipopolysaccharide. Activation of the TLR pathway results in increased AXL expression and initiates a negative feedback loop by forming a complex with the IFN- α/β receptor (IFNAR) [43]. Signaling through AXL-IFNAR induces the expression of suppressor of cytokine signaling (SOCS) 1 and 3, which in turn inhibits proinflammatory cytokine release and promotes immunosuppression to maintain tissue homeostasis. Interestingly, Villani et al. discovered a new subset of DCs characterized by the expression of AXL and sialic acid-binding Ig-like lectin 6 (SIGLEC-6), termed AS DCs [46]. This subset was identified using single-cell RNA sequencing and showed a unique gene expression profile distinct from conventional DCs and

plasmacytoid DCs. The AS DC subset exhibits high levels of genes associated with type I IFN responses, are proficient in antigen presentation and demonstrate strong responses to viral infections. The study suggested that AS DCs might originate from a common progenitor shared with other DC types but diverge to acquire their unique features. A better understanding of these AS DCs, particularly their association with type I IFN responses and potential roles in antigen presentation, could have significant implications for immunotherapies and vaccine development, particularly in the context of viral infections and cancer immunotherapy. However, further research is needed to elucidate the precise contributions of AXL in these diverse biological contexts and to determine how therapeutic AXL targeting can be optimized to mitigate disease without compromising physiological tissue repair and immune balance.

Oncogenic role of the GAS6/AXL signaling pathway

AXL is frequently overexpressed in a variety of cancer types, and its dysregulation and activation is associated with tumor progression, poor prognosis and decreased OS [47–51]. Some of the cancers in which AXL is frequently reported to be dysregulated include breast cancer [52, 53], non-small cell lung carcinoma (NSCLC) [54–56], colorectal cancer [57], ovarian cancer [58], pancreatic cancer [59], prostate cancer [60], melanoma [61] and AML [12, 62–64]. AXL's contribution to cancer can be attributed to its ability to activate downstream signaling cascades that play a major role in cancer cell survival, proliferation, migration/invasion, EMT, angiogenesis, and resistance to conventional therapies, as previously summarized by others [51, 65, 66]. AXL activation can drive cancer cell survival and proliferation through modulation of different signaling pathways, including the PI3K/AKT/mammalian target of rapamycin (mTOR), JAK/STAT, nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), and rat sarcoma virus/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase/extracellular signal-regulated kinases (RAS/RAF/MEK/ERK) pathways [67–69]. It has been found that AXL promotes tumor cell survival by regulating NF κ B nuclear translocation, decreasing the activity of pro-apoptotic proteins (BAD and caspase-3) and enhancing the expression of anti-apoptotic markers (survivin, BCL-2 and BCL-XL) (Fig. 1) [70–72].

In the context of cancer cell migration and invasion, it has been shown that GAS6-induced AXL activation promotes membrane protrusions, cell motility, actin cytoskeletal remodeling, cell spreading and regulation of lysosome peripheral distribution, mainly by PI3K/AKT, MAPK/ERK, JAK/STAT and Ras-related C3 botulinum toxin substrate 1 (RAC1) activation [73–75]. In addition, AXL-induced ERK signaling contributes to

enhanced cancer cell migration/invasion by promoting the expression of matrix metalloproteinases (MMPs), which degrade the ECM, facilitating cell movement [76]. AXL activation is also intimately linked with the induction of EMT, a cellular program that endows epithelial cells with mesenchymal characteristics, promoting increased motility and invasion. Through downstream effectors such as SNAIL, SLUG, and TWIST, AXL signaling represses the expression of epithelial markers (e.g., E-cadherin) and upregulates mesenchymal markers (e.g., N-cadherin, vimentin), facilitating the transition to a more invasive phenotype [47, 77, 78].

Finally, the GAS6/AXL axis also plays a significant role in promoting angiogenesis through several mechanisms, including the induction of pro-angiogenic factors (e.g., VEGF, ANG2) and stimulation of endothelial cell function [79, 80].

Taken together, AXL's ability to drive multiple oncogenic processes makes it an attractive therapeutic target, especially in cancers characterized by poor prognosis and drug resistance. However, understanding the context-specific roles of AXL in different cancer types, its contribution to immune evasion, and how it interacts with other signaling pathways under therapeutic pressure, awaits further investigation. This could optimize current AXL-targeted therapies, especially in combination with other treatments, while considering the broader implications for normal tissue homeostasis and potential adverse effects.

Immunoregulatory function of AXL in cancer

Besides its direct oncogenic function, AXL expression in the tumor microenvironment (TME) contributes to the creation of an immunosuppressive niche, further supporting tumor growth and therapy resistance. For example, Holtzhausen et al. demonstrated that AXL was significantly upregulated on immunosuppressive myeloid-derived suppressor cells (MDSCs) in BRAF^{V600E}/PTEN deficient melanoma tumor-bearing mice [81]. Moreover, MDSCs from tumor-bearing AXL knock-out (KO) mice failed to induce T cell suppression and migrated poorly to tumor-draining lymph nodes. A similar effect was observed upon treatment with the pan-TAM inhibitor UNC4241, which also reduced the MDSC suppressive capacity and subsequently increased CD8⁺ T cell infiltration [81].

In addition, AXL and GAS6 are both described to drive tumor-associated macrophage polarization towards an immunosuppressive, pro-tumoral, M2-like phenotype via the AXL/PI3K/AKT/NFκB pathway [82–85]. These M2 macrophages are associated with immunosuppression and tissue remodeling, mainly through secretion of anti-inflammatory cytokines and growth factors that support tumor growth and suppress immune responses.

Tumor-associated macrophages with high AXL expression produce increased levels of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), which inhibit the activation and proliferation of effector T cells and promote the expansion of regulatory T cells (Tregs). Increased M2 polarization and AXL expression was observed upon incubation of THP-1 macrophages with tumor cell derived conditioned medium, while AXL inhibition abrogated M2 polarization. In the CT26 murine colon carcinoma model, AXL inhibition by SLC-391 decreased tumor growth and increased the ratio of M1/M2-polarized macrophages [86]. Notably, a study by Goyette et al., using the HER2⁺ Neu FVB/NJ (MMTV-NeuNDL2-5) and NIC (MMTV-NeuNDL2-5-IRES-Cre) mouse models of breast cancer, demonstrated reduced HER2 and HIF-1α levels in AXL KO mice, especially upon hypoxia [87]. AXL was essential for HIF-1α expression during hypoxic stress, and its inhibition disrupted the hypoxic response. This suggests that AXL influences HIF-1α expression, particularly in vivo, affecting the TME and metastasis. Moreover, AXL KO had a direct impact on the activity and composition of immune cells. More specifically, a decreased infiltration of pro-tumoral CD206⁺ macrophages, neutrophils and immunosuppressive Tregs, and an increased infiltration of cytotoxic NK cells, CD45⁺ immune cells, CD8⁺ T cells, CD4⁺ T cells, and I-A/I-E⁺ (MHC class II⁺) macrophages was found in AXL KO tumors compared to AXL^{+/+} tumors.

Besides its expression on myeloid cell types, AXL was also detected on NK cells and identified as a critical target of the E3 ubiquitin ligase CBL-B (casitas B-lineage lymphoma-b) in B16F10 melanoma and 4T1 mammary tumors [88]. Genetic ablation of CBL-B or pharmacological inhibition of AXL using LDC1267 resulted in enhanced NK cell cytotoxicity, mainly by increased degranulation and the release of cytotoxic granules such as perforin and granzyme B, which are essential for the destruction of tumor cells. AXL inhibition also led to elevated secretion of pro-inflammatory cytokines, particularly IFN-γ, which is crucial for orchestrating anti-tumor immune responses. The enhanced secretion of IFN-γ highlights the broader immune-activating potential of AXL targeting in the tumor microenvironment. By boosting both direct cytotoxic activity and inflammatory signaling, AXL inhibition amplifies the anti-tumor effectiveness of NK cells. These findings were further supported by in vivo experiments, where LDC1267 treatment significantly reduced metastatic spread in murine models of melanoma and mammary cancer, emphasizing the therapeutic potential of modulating AXL activity in cancer immunotherapy. Previous work by Terry et al. in 2019 confirmed this by demonstrating that AXL expression is correlated with increased resistance to both NK

cell- and CD8⁺ T cell-mediated cancer cell killing [89]. AXL inhibition elicited a robust immune response, characterized by increased infiltration of CD8⁺ T cells and NK cells into the TME. This resulted in a heightened state of immune activation, where NK cells exhibited enhanced cytotoxic activity against mesenchymal lung cancer cells, mainly by increased expression of activation markers and cytolytic granules, such as perforin and granzyme B.

GAS6-AXL signaling was also found to affect the Treg population. GAS6 could enhance the suppressive abilities of Tregs both in vitro and in vivo, primarily through the activation of AXL [90]. This interaction between GAS6 and AXL increased the expression of forkhead box P3 (FOXP3) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which contribute to the suppressive function of Tregs. Using an AXL KO breast cancer model (MMTV-PyMT), Aguilera et al. observed multiple changes in the TME composition, including an increased amount of antigen-presenting myeloid DCs (CD11c⁺, MHC-II⁺) and an elevated CD8⁺ T cell infiltration [91]. This correlated with a decreased secretion of myeloid supportive cytokines (CSF1, CSF2, and CSF3), chemoattractants (CCL3, CCL4, and CCL5) and NFκB related cytokines (IL-6, TNF-α, and IL-1α).

AXL has also been implicated in the regulation of immune checkpoint molecules, including PD-L1 (CD274, PDCD1LG1 or B7-H1), on tumor cells or immune cells. Studies have shown that AXL activation can lead to the upregulation of PD-L1 expression, which in turn can inhibit the immune response by engaging PD-1 receptors on T cells and impair their activity. While the PD-L1/PD-1 axis can sometimes predict responses to anti-PD-1 therapy, its regulation is complex and influenced by various oncogenic events that enable tumor immune evasion. Research has shown that in carcinoma cells like MDA-MB-231, HeLa, and MCF7, hyperactive MERTK and AXL signaling upregulates PD-L1 expression, especially in the presence of phosphatidylserine (PS)-presenting apoptotic cells or PS-derived vesicles, partly through the PI3K/AKT pathway [92]. Boshuizen et al. reported high PD-L1 levels in AXL-expressing tumors in the preclinical human melanoma (BLM, SkMel-147) and lung cancer (LCLC-103 H) models [93]. In the study of Sadahiro et al., AXL inhibition using bemcentinib in murine glioblastoma resulted in decreased PD-L1 and increased PD-L2 (CD273, PDCD1LG2 or B7-DC) expression [94]. Furthermore, PD-L1 was predominantly expressed on tumor cells (CD45⁻) and myeloid cells (CD45⁺/CD11b⁺), though the specific impact of AXL targeting on these subsets requires further investigation. These findings collectively suggest a consistent association between AXL expression and PD-L1, contributing to the suppression of antitumor immune responses.

In addition, Aguilera et al. demonstrated that the loss of AXL in the PyMT breast cancer model not only altered the immune TME, but also increased the levels of mouse MHC class I (H-2K^b) [91]. Cancer cells typically display tumor-specific antigens or neoantigens on MHC-I molecules, which interact with CD8⁺ T cells, promoting cytotoxic T lymphocyte activity and cell-mediated lysis through the perforin/granzyme pathway or apoptosis pathways mediated by tumor necrosis factor (TNF), FAS, and TNF-related apoptosis-inducing ligand (TRAIL). Therefore, partial or complete loss of MHC-I expression can lead to tumor immune escape. Other studies reported a correlation between high AXL expression and lower MHC-I levels in lung carcinoma cell clones, though AXL inhibition did not upregulate MHC-I [89]. Notably, it did increase the expression of genes involved in antigen processing and presentation, such as *Tap1*, *Tapbp*, and *Erap2*. Further supporting the AXL/MHC-I link, a study analyzing 94 melanoma tumors, at baseline and after progression under PD-1 inhibitor treatment, found that MHC-I downregulation was associated with PD-1 inhibitor resistance [95]. Additionally, the study found associations with SNAIL upregulation and CAF signatures. TGF-β was shown to promote the expansion of AXL^{high} tumor cells and inhibit MHC-I expression, even in the presence of IFN-γ, thereby facilitating immune evasion [95]. This aligns with previous findings in prostate [96] and lung cancer cells [97], where TGF-β was identified as a repressor of MHC-I expression. Blocking TGF-β signaling could potentially overcome this immunosuppressive barrier and enhance immune responses.

Taken together, these studies across diverse models have delineated potential mechanisms underlying AXL-mediated immunosuppression, including reduced tumor antigen presentation, attenuated pro-inflammatory cytokine cascades, disrupted immune cell infiltration and increase immune checkpoint expression. While some similarities exist in reported cytokine profiles, it's crucial to acknowledge potential differences in the immune cell populations affected, owing to tumor model-specific contexts or disparities in experimental methodologies. In essence, GAS6/AXL signaling emerges as a driver of macrophage and MDSC infiltration, and a reduced abundance of mature DCs, NK cells, as well as CD4⁺ and CD8⁺ T lymphocytes. This comprehensive understanding underscores the pivotal role of AXL in shaping immune responses and highlights its potential as a therapeutic target in immunomodulatory strategies against cancer and other immune-related disorders. However, the exact mechanisms by which AXL modulates immune checkpoints and how it can be best targeted in combination with immunotherapies is yet to be determined. Addressing this could unlock new therapeutic avenues to

enhance immune responses and overcome resistance in cancer treatment.

AXL in Acute myeloid leukemia

AXL expression and its prognostic value in AML

In 1991, O'Bryan et al. reported the finding of an unexplained transforming gene in two chronic myeloid leukemia (CML) patients, marking the first discovery of AXL for the diagnosis of a chronic myeloproliferative disease (CMPD) [14, 39]. Three years later, AXL was linked for the first time to AML, when a study by Neubauer et al. showed that AXL could be detected primarily in cells derived from myeloid versus lymphoid malignancies [98]. More specifically, AXL mRNA levels were observed in hematopoietic CD34⁺ progenitor and BM stromal cells (BMSC), and to a lesser extent in peripheral blood monocytes in 59% of patients with myeloproliferative disorders (39/66 cases). In 1999, a multicenter trial of the Swiss group for Clinical Cancer Research revealed that AXL expression in AML was associated with adverse prognosis but is not confined to a single subclass of the French-American-British (FAB) classification [62]. AXL expression was reported in 35% of the AML patients (19/54 cases) and was associated with a worse progression-free survival (PFS) and OS. Interestingly, a correlation was found between AXL and BCL-2 expression levels, and AXL transcript numbers were also higher in AML patients with high CD34 expression. No other correlations based on patient's age, FAB category, or presence of extramedullary disease were found.

Using 112 samples of cytogenetically normal AML patients, Ben-Betalla et al. observed AXL and GAS6 expression in 57% and 90% of the patients, respectively [12]. Moreover, it was found that patients expressing AXL above the median correlated with a worse OS, while GAS6 lacked prognostic value. The latter was refuted in a study by Whitman et al. which investigated the prognostic relevance of GAS6 expression in 270 adults with de novo cytogenetically normal AML [11]. They were the first to report that GAS6 expression in patients, especially those aged ≥ 60 years, predicted failure to achieve CR and was correlated with a shorter disease-free survival and OS. Additionally, Yang et al. reinforced the prognostic significance of GAS6 by demonstrating that higher GAS6 expression correlated with shorter event-free survival and OS in AML patients undergoing allogeneic HSC transplantation [99]. Consistently, the study of Tirado-Gonzalez et al. revealed that high GAS6 expression correlated with poor AML patient outcomes and that patient-derived leukemic cells enhanced GAS6 expression in CD14⁺ monocytes from healthy donors [100]. Collectively, these studies underscore the significance of GAS6/AXL signaling in AML progression and its potential as a prognostic biomarker. The correlation

between AXL and BCL-2, as well as the enrichment of AXL in CD34⁺ progenitors, points to its involvement in maintaining leukemic stem cell (LSC) populations, which may contribute to disease persistence and relapse. Understanding the precise mechanisms by which AXL and GAS6 promote leukemogenesis and resistance, especially in the context of genetic and molecular heterogeneity in AML needs clarification. Moreover, monitoring AXL expression in cancer patients will allow oncologists to identify which patient subgroups would benefit most from AXL-targeted therapies and could pave the way for a more personalized and effective treatment strategy for, among others, AML patients.

AXL signaling in leukemic stem cells and AML cells in the BM niche

Although GAS6 is only expressed at low levels in AML cells, Ben-Betalla et al. identified a paracrine interaction between AML cells and GAS6-expressing BMSC. In this paracrine loop, AML cells induce GAS6 expression in BMSC, which in turn increases AXL activation and tumor cell survival [12]. Using bemcentinib, it has been found that blocking GAS6/AXL interaction attenuated AKT and MAPK signaling in MV4-11 AML cells *in vitro*. Furthermore, increased protein levels of the pro-apoptotic p53 upregulated modulator of apoptosis (PUMA) and decreased protein levels of BCL-2, p-AKT and p-ERK levels were observed upon bemcentinib treatment of AML cells [12]. In an indirect way, knock-down of ALKBH5, a N⁶-methyladenosine (m⁶a) demethylase, was found to downregulate AXL expression and reduced the phosphorylation of downstream signaling pathways, including p-SRC, p-AKT, p-ERK1/2, p-STAT3, and p-PLC γ (phospholipase C gamma), which were rescued upon restoration of ALKBH5 expression [101].

Besides its oncogenic activity in AML cells, AXL was found to contribute to AML progression and relapse by supporting the maintenance and survival of LSCs through the lysine demethylase 4 C (KDM4C)-ALKBH5-AXL signaling axis [101]. LSCs are a subset of leukemic cells characterized by their self-renewal capacity and typical resistance to conventional chemotherapies. KDM4C expression in LSCs was found to increase chromatin accessibility by reducing H3K9me₃ and recruiting MYB and Pol II to the ALKBH5 promoter. ALKBH5 subsequently promoted the expression of AXL and further activated downstream signaling pathways including PI3K, MAPK, JAK/STAT, and NF κ B, this way contributing to LSCs survival and AML progression. Furthermore, the study of Niu et al. revealed significantly elevated AXL levels in CD34⁺ stem/progenitor cells from AML patients at diagnosis [64]. Interestingly, higher AXL/GAS6 levels could be detected in stem cell-enriched Lin⁻CD34⁺CD38⁻ cells from AML patients harboring mixed

lineage leukemia (MLL) fusions compared to non-MLL samples. While all these data implicate a function of AXL in LSC survival, the impact of AXL-targeting compounds on this specific cell population, which are crucial for long-term disease control, remains unclear. Additionally, understanding the broader effects of AXL-targeting strategies within the leukemic microenvironment could contribute to more effective therapies that address both tumor and microenvironmental survival signals.

AXL as a regulator of AML cell drug resistance

The development of drug resistance in leukemia cells constitutes the major reason for treatment failure in AML patients. In solid tumors as well as in hematological malignancies, AXL expression has been linked with increased resistance to chemotherapy, immunotherapy and targeted therapies [102–107]. In 2008, Hong et al. compared the protein tyrosine kinase expression in drug-sensitive, before the administration of chemotherapy, and drug-refractory samples from the same AML patient [72]. AXL was found to be consistently overexpressed in the drug-resistant AML samples compared to the drug-sensitive AML samples. The effect of the chemotherapeutics doxorubicin, VP16 and cisplatin on AXL expression was studied using U937 AML cells and demonstrated chemotherapy-induced AXL upregulation and phosphorylation *in vitro*. Moreover, it was found that both GAS6 and AXL were required to induce chemotherapy resistance and was associated with increased expression of BCL-2 and TWIST. These findings were further supported by Ben-Betalla et al., who demonstrated that the chemotherapeutics cytarabine and doxorubicin could upregulate AXL expression in GAS6⁺ AML cell lines MV4-11 and OCI-AML5, while this effect was absent for the GAS6⁻ cell line HL60. Combining chemotherapeutics with bemcentinib significantly reduced the growth of MV4-11 cells *in vitro* and *in vivo*, while no additive effects could be observed using AKT or MAPK inhibitors in combination with bemcentinib. More recently, our group also demonstrated that Fc-conjugated AXL-targeting single domain antibodies (sdAbs) acted synergistically in combination with the standard-of-care agent cytarabine in THP-1 and MOLM-13 AML cell lines [108]. All these data underscore the AXL-mediated impact on chemoresistance and the potential of combining AXL inhibitors and chemotherapeutics in AML patients.

In addition to its role in chemoresistance, AXL overexpression also mediates resistance to various targeted therapies, including FLT3-targeted therapies [109]. In 2009, a study by Park et al. reported that AXL is crucial for optimal signaling and biological activities of c-Kit in human CD34⁺ hematopoietic progenitor cells [110]. As c-Kit is a RTK from the same type III RTK family as FLT3, these data suggested that AXL could play a role in

the regulation of FLT3 signaling. Further studies demonstrated that AXL activation contributed to a constitutive FLT3-ITD phosphorylation and activation [111]. FLT3 inhibitors midostaurin (PKC412) and quizartinib (AC220) enhanced AXL, ERK and AKT phosphorylation, and also STAT5 in the case of midostaurin. Using primary blasts of FLT3-ITD⁺ AML patients, it was found that midostaurin-sensitive cells harbored little phosphorylated AXL, while most midostaurin-resistant AML cells possessed a significantly higher level of AXL phosphorylation. Treatment of midostaurin-resistant MOLM-13 (FLT3-ITD⁺) AML cells with the AXL inhibitor TP-0903 or soluble AXL chimeric protein Axl-Fc restored sensitivity to both midostaurin and quizartinib, suggesting an important role of AXL in the resistance of FLT3-ITD⁺ AML cells against the FLT3 inhibitors. Notably, Axl-Fc was also able to inhibit cell growth and induce cell-cycle arrest and apoptosis in MV4-11 and MOLM-13 AML cells. A study by Dumas et al. provided evidence that stromal cell-secreted GAS6 activated AXL and contributed to AML progression and resistance to the FLT3-inhibitor quizartinib [112].

Although AXL expression is not directly associated with resistance to BCL-2 inhibitors, a study by Niu et al. revealed a strong synergistic effect of the BCL-2 inhibitor venetoclax and the AXL inhibitor SLC-391 in primary AML patient samples and patient-derived xenograft models [64]. These effects were mainly attributed to a significant downregulation of oxidative metabolism. Additive effects were also observed by our group using the combination of venetoclax and Fc-conjugated AXL-targeting sdAb20 in THP-1 and MOLM-13 cells [108]. The combination of AXL inhibitor ONO-7475 and venetoclax demonstrated significant synergistic effects in FLT3-ITD⁺ MV4-11 and MOLM-13 AML cell lines, enhancing apoptosis and reducing cell viability compared to single agent therapy [113]. Using primary FLT3-ITD AML samples, Post et al. showed that ONO-7475 as a monotherapy showed limited efficacy, and venetoclax alone had minimal impact on apoptosis in certain primary samples, whereas their combination synergized to activate apoptosis. This underscores the importance of combinatorial approaches to effectively target AML with heterogeneous responses.

AXL-mediated immunosuppression in AML

Studies demonstrating that AXL inhibitors restore drug sensitivity, especially in combination therapies, suggest that targeting AXL could mitigate resistance and improve treatment outcomes. However, the precise mechanisms through which AXL influences various resistance pathways, particularly within the bone marrow microenvironment, and its broader effects on AML cell metabolism and immune evasion remain areas requiring further

investigation. In their comprehensive study, Tirado-Gonzalez et al. conducted an in-depth investigation into the effects of GAS6/AXL pathway inhibition on leukemia cell growth and the immune response, using the ASXL1 and MLL-ENL AML mouse models [82]. The researchers employed a GAS6-deficient immunocompetent mouse model (GAS6^{-/-}) and observed a substantial reduction in leukemic burden and significantly prolonged survival rates upon GAS6 absence. These beneficial effects were not observed in immunocompromised mice, underscoring the critical role of the immune system in mediating these effects. To dissect the contributions of different immune cell types, the study employed conditional KO models using NSG mice. While selective AXL deletion in DCs, using a CD11c-eGFP-Cre line, did not result in increased anti-leukemic immunity, depletion of AXL in macrophages using Csf1r-Cre⁺ Axl^{fl/fl} mice did increase anti-leukemic effects and prolonged survival of AML models. This macrophage-specific AXL deletion not only prolonged survival in AML models, but also resulted in heightened activation of both T cells and NK cells, as characterized by increased production of pro-inflammatory cytokines (IL-12, TNF- α), effectively targeting and eradicating both naïve and treatment-resistant leukemia cells.

Using an anti-NK1.1 antibody, the researchers reported similar leukemic burdens in both NK-depleted and non-depleted Axl^{fl/fl} control animals, indicating functional impairment of NK cells. However, NK cell depletion in Csf1r-Cre⁺ Axl^{fl/fl} mice abolished anti-leukemic immunity and led to faster disease progression, demonstrating that AXL inhibition in phagocytes triggers a robust NK cell response that is crucial for leukemia clearance. By generating mice that lacked both CD8⁺ T cells and AXL expression in phagocytes (Csf1r-Cre⁺ Axl^{fl/fl} CD8a^{-/-} and Axl^{fl/fl} CD8a^{-/-}), it was shown that the survival benefit conferred by AXL-deficient phagocytes persisted even in the absence of CD8⁺ T cells. This indicates that other immune cells play a significant role in mediating the effects of AXL inhibition.

To further assess the therapeutic potential of AXL inhibition, leukemia-bearing mice were treated with bemcentinib on an intermittent schedule (5 days on, 2 days off). Bemcentinib significantly increased OS in the Asxl1^{-/-} model, but lost this effect in immunocompromised NSG mice, which is in line with the drug's expected impact on AXL-positive immune cells.

AXL-targeted therapies in preclinical and clinical evaluation for AML

The recognition of AXL as a critical mediator of AML pathogenesis has spurred the development of AXL-targeted therapies aimed at disrupting AXL signaling and sensitizing leukemia cells to conventional chemotherapy.

Small molecule selective inhibitors (Table 1), multitargeted inhibitors (Tables 1 and 2), antibody-drug conjugates (ADC) (Table 3) and anti-AXL-Fc fusion proteins (Table 3) have shown promising preclinical efficacy towards AML, attenuating leukemic cell proliferation, inducing apoptosis, and enhancing the efficacy of standard chemo- and immunotherapeutic agents. Additionally, combinatorial approaches using AXL inhibitors in conjunction with targeted agents against other dysregulated pathways in AML, such as FLT3 inhibitors or BCL-2 inhibitors, hold potential for overcoming therapeutic resistance.

Small molecule inhibitors

The past years, various small molecule inhibitors targeting AXL have been evaluated for the treatment of AML (Tables 1, 2 and 3; Fig. 2). Interestingly, most of these inhibitors are multikinase inhibitors and often exhibit broad-spectrum activity, targeting kinases such as TAM family members TYRO3 and MERTK; as well as other RTKs such as vascular endothelial growth factor receptor (VEGFR), MET, FLT3, recepteur d'origine nantais (RON), and AURORA A/B in addition to AXL, which can contribute to their therapeutic efficacy in AML. AXL inhibitors can be classified into two main categories, namely type I and type II inhibitors. Type I inhibitors bind to the active "aspartate-phenylalanine-glycine (DFG)-in" conformation of the kinase, interacting with the ATP-binding site when the kinase is in its "on" state. Conversely, type II inhibitors prefer the inactive DFG-out conformation of the kinase, targeting an adjacent allosteric site that is exposed only when the kinase is "off" [114, 115]. This distinction is crucial as it influences the binding affinity, selectivity, and overall therapeutic potential of the inhibitors. Several AXL inhibitors have shown promise in preclinical studies and have advanced into different stages of clinical investigation [116–118]. These inhibitors, by targeting multiple pathways and conformations, hold potential for enhancing therapeutic outcomes and overcoming resistance mechanisms in AML treatment.

Type I AXL inhibitors

Bemcentinib (BGB324, R428; Rigel Pharmaceuticals/BerGenBio)

Bemcentinib is a first-in-class, oral, specific and highly selective AXL kinase inhibitor and is by far the most advanced anti-AXL therapeutic agent [119]. The activity of bemcentinib is limited to the tyrosine kinase subfamily, with the highest inhibitory potency for AXL among all 133 kinases [120]. It has been reported that bemcentinib blocks the catalytic activities of AXL, at nanomolar concentrations (IC₅₀=14 nM), reduces AXL and p-AXL levels, induces the accumulation of autophagosomes and lysosomes, blocks lysosomal acidification and recycling,

Table 1 Overview of type I AXL inhibitors and their current status in clinical trials for AML

Drug	Developer	Target(s) ^a	IC ₅₀ for AXL	Clinical Trial No. ^b	Phase of approval	Indications	Monotherapy/ combinations	Status
BGB324 (R428/Bemcentinib)	Rigel Pharmaceuticals/BerGen Bio	AXL (selective)	14 nM (in vitro) 14 nM (in cells)	NCT02488408	Ib/II	AML, MDS	± Cytarabine/ Decitabine	Unknown
TP-0903 (Dubermininib)	Tolero Pharmaceuticals	AXL, FLT3	27 nM (in vitro)	NCT03013998	Ib/II	AML	Biomarker-based multidrug therapy	Recruiting
Gilteritinib (ASP2215, Xospata®)	Astellas Pharma/Kotobuki Pharmaceutical	FLT3, AXL	0.73 nM (in vitro)	NCT02421939	III	R/R FLT3-mutated AML	Gilteritinib vs. LoDAC, FLAG-IDA, MEC & Azacitidine	Active, not recruiting
				NCT02752035	III	De novo FLT3-mutated AML	± Azacitidine	Active, not recruiting
				NCT03182244	III	R/R FLT3-mutated AML	Gilteritinib vs. LoDAC, MEC & FLAG	Active, not recruiting
				NCT04293562	III	De novo FLT3-mutated AML	Multidrug treatment	Active, not recruiting
				NCT05177731	III	AML	Multidrug treatment	Active, not recruiting
				NCT02115295	II	AML, high-risk MDS or Blastic Phase CML	Multidrug treatment	Recruiting
				NCT05955261	II	Pediatric AML	Multidrug treatment	Recruiting
				NCT06022003	II	R/R FLT3-mutated AML	+ Azacitidine	Recruiting
				NCT06221683	II	AML	Multidrug treatment	Recruiting
				NCT06317649	II	De novo FLT3-mutated AML	+ Venetoclax & Azacitidine	Not yet recruiting
				NCT03013998	Ib/II	AML	Biomarker-based multidrug	Recruiting
				NCT05279859	Ib/II	R/R FLT3-mutated AML	+ ERAS-007/ ERAS-601	Withdrawn
				NCT05028751	Ib/II	R/R FLT3-mutated AML	+ Lanraplenib	Terminated
				NCT02310321	I/II	De novo AML	+ Cytarabine/ Idarubicin	Active, not recruiting
				NCT04140487	I/II	FLT3-mutated AML or MDS	+ Venetoclax & Azacitidine	Active, not recruiting
				NCT04240002	I/II	R/R FLT3-mutated AML	+ FLAG	Recruiting
				NCT05010122	I/II	R/R FLT3-mutated AML or high-risk MDS	+ Venetoclax/ Decitabine & Cedazuridine	Recruiting
				NCT05520567	I/II	De novo FLT3-mutated AML	+ Venetoclax/ Azacitidine	Recruiting
				NCT06235801	I/II	R/R FLT3-mutated AML	+ Mometinib	Recruiting
				NCT04336982	Ib	De novo or R/R AML	+ CC-90,009/ Venetoclax/ Azacitidine	Terminated
				NCT05010772	Ib	AML in remission	+ Decitabine & Cedazuridine	Recruiting
				NCT04655391	Ib	AML Post-AHCT	+ Glasdegib	Withdrawn
				NCT05330377	Ib	R/R FLT3-mutated AML	+ Mitoxantrone/ Cladribine/ Cytarabine/ Filgrastim	Withdrawn
				NCT05756777	Ib	R/R FLT3/IDH1 or FLT3/IDH2-mutated AML	+ Ivosidenib/ Enasidenib	Recruiting
				NCT05024552	I	R/R FLT3-mutated AML	+ Vyxeos	Recruiting

Table 1 (continued)

Drug	Developer	Target(s) ^a	IC ₅₀ for AXL	Clinical Trial No. ^b	Phase of approval	Indications	Monotherapy/ combinations	Status
				NCT05546580	I	R/R FLT3-mutated AML	+ ladamostat	Recruiting
				NCT06222580	I	R/R FLT3-mutated AML with MLL or NPM1 mutation	+ Revumenib	Recruiting
				NCT06001788	I	R/R AML with KMT21- or NPM1-mutation	+ Zifomenib	Recruiting
				NCT05312112	Observational	AML	± Venetoclax	Recruiting
				NCT04691648	Observational	R/R FLT3-mutated AML	Monotherapy	Recruiting
				NCT06265545	Platform trial	R/R FLT3-mutated AML	+ Ivosidenib/ Venetoclax/ Selinexor	Not yet recruiting

Abbreviations: MDS: Myelodysplastic syndromes, R/R: Relapsed or refractory, FLT3: Fms-like tyrosine kinase 3, AML: Acute myeloid leukemia, IDH1/2: Isocitrate dehydrogenase 1/2, NPM1: Nucleophosmin 1, ALL: Acute lymphoblastic leukemia

^aIn the order of inhibition potency

^bAll the relevant information of clinical trials can be found on the public clinical trial registry website (clinicaltrials.gov)

and increases apoptosis of tumor cells [121]. In addition, Ben-Betalla et al. reported that, when used alone or in combination with cytotoxic agents, bemcentinib abrogated the AKT and MAPK pathways by upregulating PUMA and suppressing BCL-2 [12]. Bemcentinib was the first AXL-specific inhibitor to enter clinical investigation and is currently in phase I/II clinical trials, either alone or in combination with other therapies, for the treatment of AML, myelodysplastic syndromes (MDS), triple-negative breast cancer (TNBC), glioblastoma, NSCLC, metastatic melanoma and malignant mesothelioma (NCT03965494, NCT02922777, NCT02872259, NCT03184558, NCT03184571, NCT02488408, NCT02424617, NCT03824080 and NCT03654833). Loges et al. reported that bemcentinib exhibited the potential for safe administration over prolonged periods, effectively suppressing AXL signaling and demonstrating anti-leukemic activity [122]. Bemcentinib was well tolerated as a monotherapy and in combination with low-dose cytarabine (LDAC) or decitabine in the phase Ib/II study BGBC003 (NCT02488408), after which bemcentinib was given FDA fast-track designation for the treatment of elderly patients with relapsed/refractory (R/R) AML [123, 124]. Further evaluation in the BERGAMO phase II trial (NCT03824080) showed bemcentinib's moderate efficacy in high-risk R/R MDS/AML, with a 24% overall response rate, and a higher response in MDS patients (44%) compared to AML patients (11%) [125]. Although exploratory analysis suggested that patients with STAG2 mutations may respond better to bemcentinib, further research is needed to refine patient selection and improve outcomes, potentially by combining bemcentinib with other therapies.

Dubermatinib (TP-0903; Tolero Pharmaceuticals)

TP-0903 is a potent AXL inhibitor with high-affinity, oral bioavailability and concurrent FLT3 inhibitory activity. With an in vitro IC₅₀ of 27 nM [126, 127], TP-0903 disrupts AXL phosphorylation, reverses EMT, and increases the depletion of anti-apoptotic proteins MCL-1, X-linked inhibitor of apoptosis protein (XIAP), and BCL-2, fostering dose-dependent apoptosis of primary chronic lymphocytic leukemia (CLL) B-cells, even in cases with adverse prognostic factors like 17p/p53 deletions or prior exposure to agents like ibrutinib [128, 129]. TP-0903 also demonstrated promising efficacy against *de novo* and drug-resistant FLT3-ITD⁺ AML, both in vitro and in vivo [130]. Furthermore, Park et al. reported that TP-0903 resensitized MOLM-13 FLT3-ITD⁺ AML cells to the FLT3-inhibitors midostaurin and quizartinib [111]. However, as TP-0903 was found to also target aurora kinase and JAK2, it could not be ruled out that abrogation of FLT3-ITD⁺ AML resistance was due to off-target activities of TP-0903. Jeon et al. observed a

Table 2 Overview of type II AXL inhibitors and their current status in clinical trials for AML

Drug	Developer	Target(s) ^a	IC ₅₀ for AXL	Status
Cabozantinib (XL184, Cabometyx, BMS-907351, Cometriq®)	Exelixis/Ipsen company	VEGFR2, MET, RET, KIT, AXL, FLT1/3/4	7 nM (in vitro), 42 nM (in cells)	Terminated phase Ib trial ^b (NCT03878524)
Foretinib (XL880, EXEL-2880, GSK1363089)	GSK	MET, VEGFR2, TIE-2, VEGFR3, RON, AXL	11 nM (in vitro), < 100 nM (in cells)	Preclinical
Sitravatinib (MGCD516)	Mirati Therapeutics Inc.	DDR2, EPHA3, AXL, MER, VEGFR3	1.5 nM (in vitro), 250–800 nM (in cells)	Preclinical
Rebastinib (DCC-2036)	Deciphera Pharmaceuticals LLC	ABL, FLT3, VEGFR2, TIE-2, Lyn, SRC, FGR, AXL	42 nM (in vitro)	Preclinical

^aIn the order of inhibition potency^bAll the relevant information of clinical trials can be found on the public clinical trial registry website (clinicaltrials.gov)**Table 3** Other AXL inhibitors, anti-AXL monoclonal antibodies and soluble receptors in preclinical development for AML

Drug	Drug class	Target(s) ^a	Clinical stage
UNC569	Small molecule inhibitor	MERTK, AXL	Preclinical
CTS2016	Small molecule inhibitor	AXL, FLT3	Preclinical
SLC-391	Small molecule inhibitor	AXL, TYRO3, MERTK	Preclinical
DAXL-88	Antibody	AXL, FLT3	Preclinical
DAXL-88-MMAE	Antibody-drug conjugate	AXL, FLT3	Preclinical
sdAb20-Fc	Single domain antibody	AXL	Preclinical
Axl-Fc	Soluble receptor	GAS6	Preclinical
MYD1-72	Decoy receptor	GAS6	Preclinical

^aIn the order of inhibition potency

correlation between TP-0903's potency and STAT, AKT, and ERK signaling, alongside cell cycle regulators in both biochemical and cellular assays [131]. Their findings demonstrated that TP-0903 exhibited *ex vivo* activity in primary AML cells characterized by recurrent mutations like MLL-PTD, ASXL1, SRSF2, and WT1, which are known to be associated with unfavorable prognosis or to contribute to clinical resistance against AML-directed therapies. In TP53 mutant AML cell lines, TP-0903 effectively inhibited cell viability (IC₅₀=12–32 nM), induced apoptosis at 50 nM, and upregulated pChk1/2 and pH2AX, indicating DNA damage induction [132]. Moreover, the combination of TP-0903 and decitabine showed additive effects *in vitro* and significantly prolonged median survival in mouse models xenografted with TP53 mutant AML compared to single-agent treatments. Clinical trials regarding TP-0903 as a monotherapy include patients with CLL, refractory solid tumors and FLT3 mutated AML (NCT03572634, NCT02729298 and NCT04518345). In addition, the ongoing Beat AML phase 1b/2 study of TP-0903 and decitabine targeting mutant TP53 and/or complex karyotype in patients with untreated AML (NCT03013998) reported a composite CR (CRh/Cri) rate of 66% achieving minimal residual

disease negativity at dose level 1, leading to selection of this dose as the recommended phase 2 dose [118].

Gilteritinib (ASP2215; Astellas Pharma/Kotobuki Pharmaceutical)

Gilteritinib is a highly specific inhibitor of FLT3, AXL and anaplastic lymphoma kinase (ALK or CD246), exhibiting significant anti-leukemic activity in patients with R/R AML. Targeting both AXL and FLT3 with gilteritinib results in tumor regression and decreased proliferation in FLT3 mutation-positive AML models, both *in vitro* and *in vivo* [133]. Dumas et al. reported that gilteritinib possessed anti-proliferative and pro-apoptotic effects on FLT3-ITD⁺ MOLM-13, MOLM-14 and MV4-11 AML cell lines [134]. These effects were associated with reduced phosphorylation of FLT3 and AXL, as well as decreased activation of downstream signaling pathways. The study indicated that dual FLT3/AXL inhibition by gilteritinib provided more effective inhibition of FLT3-ITD⁺ cells, compared to the highly selective FLT3 inhibitor quizartinib, under conditions mimicking the BM microenvironment, such as hypoxia and co-culture with BM stromal cells. However, the enhanced efficacy of gilteritinib may partly be attributed to differences in dosing, and additional studies are needed to confirm whether AXL inhibition specifically contributes to this effect. Notably, the study observed that AXL expression and its ligand GAS6 are upregulated in hypoxic conditions and in BM stromal cell co-culture.

Early phase I/II trials demonstrated the anti-leukemic efficacy and tolerable side effects of gilteritinib in R/R AML patients across the USA, Germany, Italy, and Japan (NCT02181660 and NCT02014558) [116]. The multicenter, randomized phase III ADMIRAL trial (NCT02421939) confirmed that gilteritinib significantly improves OS and CR rates compared to chemotherapy, with fewer adverse events and a comparable safety profile, in R/R AML patients with FLT3 mutations, leading its FDA approval for this indication [135–137]. Current research includes exploring gilteritinib in combination with other therapies, such as venetoclax (NCT03625505),

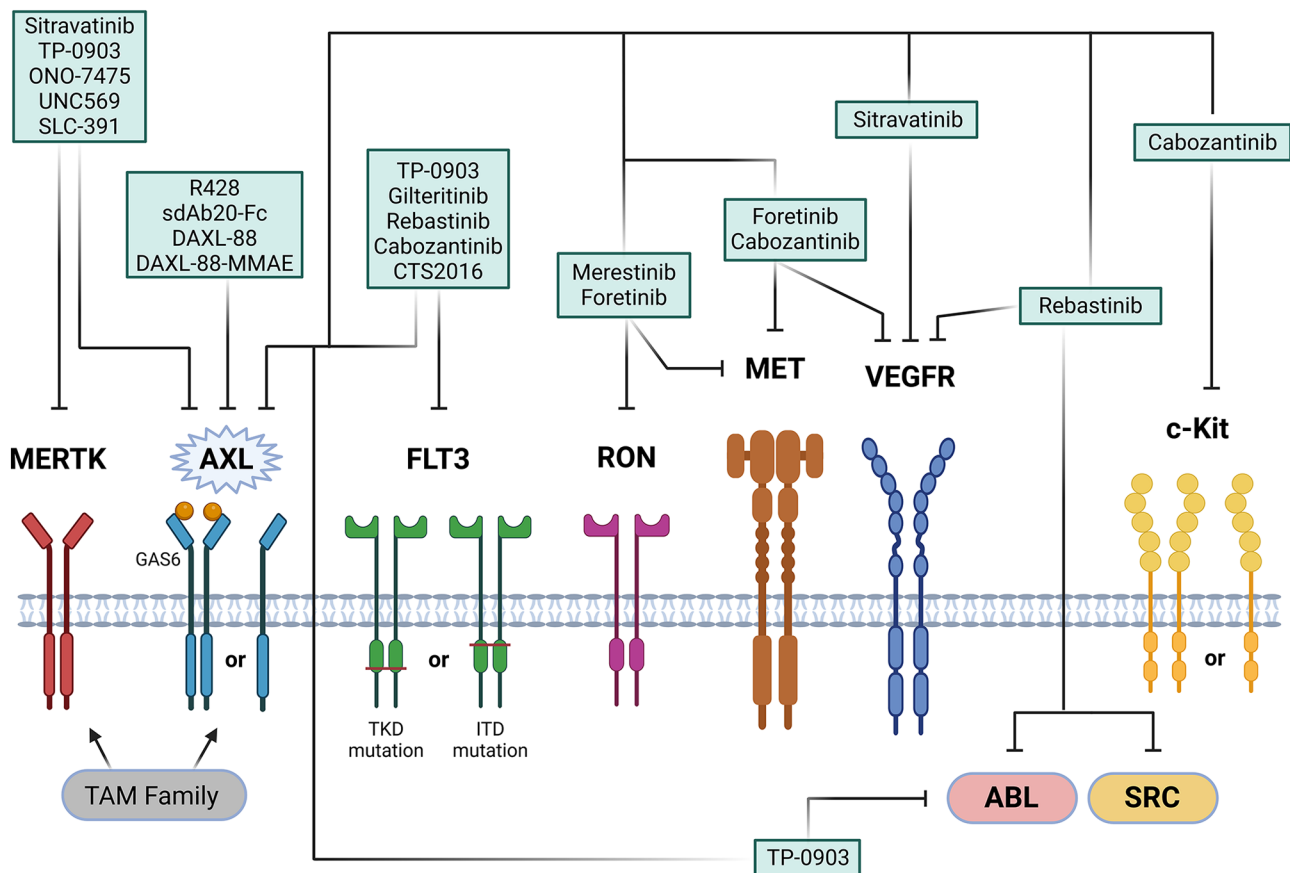


Fig. 2 AXL-targeted therapies in (pre)clinical evaluation for AML Summarizing figure of all AXL-targeted therapies in AML, including small molecule inhibitors, antibody-drug conjugates (ADCs), anti-AXL-Fc fusion proteins, and inhibitors targeting multiple receptors and/or proteins. These agents have demonstrated significant (pre)clinical efficacy by reducing leukemic cell proliferation and inducing apoptosis, thereby boosting the effectiveness of standard chemo- and immunotherapy. FLT3, FMS-like Tyrosine Kinase 3; RON (MST1R), Macrophage Stimulating 1 Receptor; MET, Mesenchymal to Epithelial Transition factor (also known as Hepatocyte Growth Factor Receptor, HGFR); VEGFR, Vascular Endothelial Growth Factor Receptor; c-KIT, v-kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog (also known as CD117); ABL, Abelson Murine Leukemia Viral Oncogene Homolog 1; SRC, Proto-Oncogene Tyrosine-Protein Kinase; TKD, Tyrosine Kinase Domain; ITD, Internal Tandem Duplication. Figure created with BioRender.com

which has demonstrated high composite CR rates, and atezolizumab (NCT03730012), which, despite an acceptable safety profile at lower doses, exhibited dose-limiting toxicities at higher doses [138, 139]. However, these findings support continued investigation into gilteritinib-based combination therapies. Therefore, gilteritinib is currently being evaluated in several clinical trials on AML patients, including in combination with gemtuzumab ozogamicin (Mylotarg[®]; NCT06022003), azacytidine (NCT06022003, NCT04140487, NCT02752035), atezolizumab (NCT03730012), ivosidenib/enasidenib (NCT05756777), iadademstat (NCT05546580), decitabine (NCT05010772), momelotinib (NCT06235801), revumenib (NCT06222580), CC-90,009 (NCT04336982), venetoclax/azacytidine (NCT05520567, NCT04140487), cytarabine/daunorubicin (Vyxeos[®]; NCT05024552) and venetoclax/decitabine (NCT05010122).

Tamnorzatinib (ONO-7475; Ono Pharmaceutical Co., Ltd.)

The dual AXL/MERTK inhibitor ONO-7475 has demonstrated efficacy in targeting FLT3-mutant AML cells by inhibiting key signaling pathways involving ERK and MCL-1, which are implicated in drug resistance against venetoclax [113]. Combination treatment of ONO-7475 with venetoclax exhibited potent cytotoxicity against FLT3-mutant AML cell lines and primary cells, including those resistant to venetoclax, particularly those overexpressing MCL-1 [140]. In vivo studies demonstrated that ONO-7475 monotherapy significantly prolonged survival in AML cell lines and PDX models [113]. In short, the combination of ONO-7475 and venetoclax surpassed the efficacy of ONO-7475 monotherapy, significantly reducing leukemic burden and extending survival in both model systems. Based on these findings, ONO-7475 entered clinical evaluation to assess its safety and tolerability as monotherapy in patients with R/R AML or MDS and to investigate its efficacy in combination with

venetoclax (NCT03176277). However, this study was terminated in May 2024 due to its ineffectiveness.

Type II AXL inhibitors

Cabozantinib (XL184; Exelixis/Ipsen company)

Cabozantinib is a non-selective multikinase inhibitor that targets multiple RTKs, including VEGFR2, c-MET, KIT (also known as CD117), RET, AXL, ROS1 and FLT3, and the related angiogenesis and metastasis processes. It has been demonstrated that cabozantinib induces apoptosis in FLT3-ITD⁺ leukemia cells in a dose-dependent manner [141]. However, FLT3-ITD lacking leukemia cell lines were resistant to cabozantinib. A clinical trial (NCT01961765) revealed favorable tolerability of cabozantinib in AML patients, with notable potency observed as an inhibitor of FLT3/ITD-altered tyrosine kinases [142]. Other phase III trials are now ongoing in, among others, differentiated thyroid cancer (NCT03690388), meningioma (NCT05425004), recurrent liver cancer post-transplant (NCT04204850), locally advanced kidney cancer (NCT04022343) and carcinoid tumors (NCT03375320).

Merestinib (LY2801653; Eli Lilly and Company/Dana-Farber Cancer Institute)

Merestinib (LY2801653) is a dual MET/AXL inhibitor that also targets RON, FLT3, MERTK, angiopoietin-1 receptor (also known as CD202B or TEK), ROS1, discoidin domain receptor family member 1/2 (DDR1/2), and the MAP kinase-interacting serine/threonine-protein kinases 1/2 (MKNK1/2) [143]. Merestinib potently blocks AXL and MET phosphorylation, and the subsequent activation of their downstream signaling molecules, and has demonstrated anti-leukemic effects by effective blockade of eukaryotic translation initiation factor 4E (eIF4E) phosphorylation in AML cells [144]. Additionally, Kosciuzuk et al. demonstrated the suppression of early leukemic progenitors derived from AML patients both in vitro and in a xenograft mouse model of AML. A single-center, nonrandomized, open-label phase I clinical trial in R/R AML patients (NCT03125239) has been completed and reported reversible grade 3 transaminase elevation as the primary dose-limiting toxicity of merestinib, either alone or in combination with LY2874455 [145]. Despite this, several patients experienced stable disease, with one achieving CR without measurable residual disease. Single agent merestinib showed safety and biological activity, with correlative studies indicating therapeutic plasma levels, effective attenuation of MET signaling, and increased hepatocyte growth factor expression in BM aspirate samples of refractory leukemia patients.

Foretinib (XL880, EXEL-2880, GSK1363089; GSK)

Foretinib is an oral multikinase inhibitor that targets several key kinases, including AXL, MET, VEGFR, ROS, RON, and TIE-2 [146]. By blocking AXL phosphorylation, foretinib effectively suppresses cell proliferation, dissemination, and survival, leading to inhibition of in vivo tumor growth [57]. In the context of AML, Wang et al. reported that foretinib exhibited superior efficacy compared to existing FLT3 inhibitors in patients with FLT3-ITD mutations [147]. Foretinib directly bound to FLT3, effectively inhibiting its signaling pathway, resulting in potent anti-proliferative and pro-apoptotic effects in AML cell lines and primary AML cells harboring FLT3-ITD mutations. Notably, foretinib also showed activity against secondary FLT3-ITD mutations resistant to quizartinib and gilteritinib. Several clinical trials for the treatment of recurrent/metastatic breast cancer, HCC, NSCLC, metastatic gastric cancer, papillary RCC and squamous cell cancer, have been conducted or are still ongoing and have reported antitumor activity of foretinib, with partial responses and stable disease [148].

Sitravatinib (MGCD516; Mirati Therapeutics Inc.)

Sitravatinib inhibits a closely related group of RTKs, including KIT, PDGFR α , PDGFR β , AXL and MET [149]. Zhang et al. demonstrated that sitravatinib reduced cell proliferation, induced cell cycle arrest, and increased apoptosis in FLT3-ITD AML cell lines [150]. In vivo studies showed superior therapeutic efficacy of sitravatinib compared to gilteritinib in MOLM-13 xenograft and BaF3-FLT3-ITD models. In addition, sitravatinib maintained potent activity against FLT3 mutation in the presence of cytokines through the robust inhibition of p-ERK and p-AKT. Additionally, leukemic blasts of patients harboring the FLT3-ITD mutation were more sensitive to sitravatinib than gilteritinib in vitro and in a PDX model.

Sitravatinib is currently undergoing clinical evaluation as a monotherapy (NCT02978859) and in combination with other therapeutics like tislelizumab and nivolumab (NCT05407519, NCT04727996, NCT05228496, NCT03906071, NCT05542342, NCT04734262, NCT04887870 and NCT04904302) in different solid tumors.

Rebastinib (DCC-2036; Deciphera Pharmaceuticals LLC)

Rebastinib, identified as a switch control inhibitor of BCR-ABL1 and FLT3 [151], was investigated in a phase 1 study in R/R CML and AML [152]. Although hematologic CR was achieved in 8/16 CML patients, including those with the T315I mutation, no responses were observed in AML patients. This insufficient clinical benefit led to the discontinuation of its development in both CML and AML. Pharmacodynamic analyses suggest that

other kinases inhibited by rebastinib, such as TIE2, may be more relevant targets.

AXL inhibitors in preclinical development

CTS2016 is a newly identified, selective, orally bioavailable small molecule inhibitor targeting AXL and FLT3 with single-digit nanomolar potency [153]. CTS2016 was evaluated both as a single agent and in combination with venetoclax or azacitidine in various *in vitro* and *in vivo* AML models and has been reported to exhibit strong growth inhibition and induced cell death in AML cell lines with FLT3 mutations. Combination treatments of CTS2016 with venetoclax or azacitidine showed enhanced therapeutic benefits compared to monotherapy, suggesting potential for use in R/R AML and MDS. Notably, CTS2016 effectively reduced tumor burden in a leukemia model with the drug-resistant FLT3-ITD-F691L mutation and displayed selectivity over other kinases, reducing off-target toxicities.

Niu et al. discovered SLC-391, a potent inhibitor targeting MERTK, AXL, and TYRO3 with IC₅₀ values of 44 nM, 9.6 nM, and 42.3 nM, respectively [64]. AML cell lines with high GAS6/AXL expression and MLL/FLT3-ITD mutations (MV4-11, MOLM-13), exhibited greater sensitivity to SLC-391 than cell lines with low GAS6/AXL expression and no MLL/FLT3-ITD mutations. Combining SLC-391 with venetoclax resulted in synergistic effects, significantly reducing cell viability, and increasing apoptosis in AML cell lines and primary patient cells, including stem cells, progenitors and myeloid blasts, as identified by multiple cell surface markers (e.g., CD47, CD44, CD99, CD123, and TIM3). *In vivo*, the combination therapy of SLC-391 and venetoclax delayed leukemia progression, prevented splenomegaly, and prolonged survival of both MV4-11 xenografted mice and AML PDX mice.

The novel MERTK and AXL inhibitor UNC569 [154, 155] has also shown promising preclinical efficacy. Koda, et al. reported suppressed cell growth and induced apoptosis of OCI-AML5 and TMD7 AML cells upon UNC569 treatment, accompanied by reduced phosphorylation of MERTK, AKT and ERK and induced cleavage of caspase-3 [156].

Anti-AXL antibodies

In 2019, Duan et al. identified the AXL-targeting antibody DAXL-88, which effectively blocked the AXL-GAS6 interaction by binding with high affinity to both human and mouse AXL proteins [157, 158]. Additionally, DAXL-88 reversed the activation of key signaling molecules, including p-AXL, p-AKT, and p-ERK, which are typically upregulated by GAS6. Building on these findings, DAXL-88 was further developed into an antibody-drug conjugate (ADC), named DAXL-88-MMAE, by linking it with

monomethyl auristatin E (MMAE), a cytotoxic agent that disrupts microtubule function. Upon binding to AXL, DAXL-88-MMAE is internalized, and the MMAE payload is released via lysosomal protease cleavage, leading to microtubule destabilization, cell cycle arrest, and apoptosis. In 2021, Liu et al. extended the study of both DAXL-88 and DAXL-88-MMAE to FLT3-ITD⁺ AML, particularly in cells resistant to the FLT3 inhibitor quizartinib [159].

Their findings revealed that these AXL-targeted agents exhibited dose-dependent cytotoxicity in both FLT3-mutant AML cell lines (MV4-11 and quizartinib-resistant MV4-11) and primary AML blast cells from FLT3-ITD⁺ patients, especially when combined with quizartinib. The observed cytotoxicity is attributed to the inhibition of AXL, FLT3, and their downstream signaling pathways, which are crucial for the survival and proliferation of these cancer cells. Liu et al. further demonstrated that DAXL-88 could overcome resistance to the FLT3 inhibitors midostaurin and quizartinib, as well as to conventional chemotherapy agents used in the clinical '7+3' induction regimen [159]. By generating chemoresistant AML cell lines, it was shown that DAXL-88 exerts dose-dependent cytotoxicity in various AML models, including FLT3-WT THP-1, FLT3-ITD⁺ MV4-11, and quizartinib-resistant MV4-11 cells. This effect is likely mediated by blockade of the interaction between AXL and FLT3, thereby inhibiting downstream AKT/ERK signaling and inducing apoptosis. Notably, DAXL-88-MMAE demonstrated even stronger growth inhibition and pro-apoptotic effects in FLT3-ITD⁺ MV4-11 cells, accompanied by a reduction in both AXL and FLT3 signaling molecules, further enhancing its therapeutic potential.

The past years, we developed alpaca-derived, small sized sdAbs (12–15 kDa), cross-reactive for mouse and human AXL protein, and evaluated their potential for the diagnosis and treatment of AML [108]. We believe that sdAbs exhibit remarkable stability, solubility, and resistance to proteases, making them promising candidates for drug development with lower immunogenicity and simplified production processes compared to conventional mAbs [160, 161]. Several anti-AXL sdAbs were characterized using ELISA, flow cytometry, surface plasmon resonance and the AlphaFold2 prediction software. SdAb20 was selected as a lead compound for diagnosis and was, for therapeutic purposes, fused to a mouse IgG2a-Fc tail (sdAb20-Fc). Biodistribution studies demonstrated the tumor-specific uptake of sdAb20 in THP-1 xenografts and immunocompetent C1498 AML mice. Importantly, the signal in tumor-bearing mice was higher compared to the background signal in naive mice, illustrating its value as a diagnostic tracer in cancer patients. Therapeutically, we found that sdAb20-Fc demonstrated

significant anti-tumor effects by inhibiting cell proliferation and cell viability in human AML cell lines and primary patient samples. Moreover, besides its clear single agent anti-tumor effect, our data also demonstrated the therapeutic potential of AXL-specific sdAb20-Fc in combination with the standard-of-care agents cytarabine and venetoclax. Interestingly, we found that cytarabine could induce AXL expression, and that the combination with sdAb20-Fc resulted in synergistic anti-AML effects. On the other hand, venetoclax had no effect on AXL expression and the combination with sdAb20-Fc resulted in additive effects in AML cell lines.

Soluble receptors

Park et al. reported that the inhibition of AXL activation using a soluble AXL chimeric protein, Axl-Fc, was able to diminish constitutive FLT3 phosphorylation in FLT3-ITD⁺ AML [109]. Furthermore, Axl-Fc disrupted the physical interaction between AXL and FLT3, inhibited cell growth, induced cell-cycle arrest and apoptosis, and alleviated the block in myeloid differentiation of FLT3-ITD⁺ AML cells in vitro. Later, it was even demonstrated that AXL inhibition with Axl-Fc substantially reduced resistance to the FLT3 inhibitors midostaurin and quizartinib using MOLM-13 FLT3-ITD⁺ AML cells [111].

Kariolis et al. developed an AXL “decoy receptor” that binds GAS6 with high affinity and effectively inhibits its function [162]. This modified AXL variant contains four mutations, enhancing its affinity for GAS6 by 80-fold. The enhanced decoy receptor, MYD1-72, has been shown to significantly reduce cell growth and induce cytotoxicity in both OCI-AML3 and MV4-11 AML cells [163].

Conclusion and future perspectives

The recognition of AXL as a critical mediator of AML pathogenesis has stimulated the development of AXL-targeted therapies aimed at disrupting AXL signaling and sensitizing leukemia cells to conventional chemotherapy. Small molecule inhibitors, monoclonal antibodies and sdAbs targeting AXL have shown promising preclinical efficacy in AML models, attenuating leukemia cell proliferation, inducing apoptosis, and enhancing the efficacy of standard chemotherapeutic agents. Additionally, combinatorial approaches with AXL inhibitors and targeted agents against other dysregulated pathways in AML, such as FLT3 inhibitors or BCL-2 inhibitors, hold potential for synergistic antileukemic effects and overcoming therapeutic resistance. In addition, recent studies also illustrated the importance of GAS6/AXL signaling in the immunosuppressive TME, fostering the evaluation of AXL-targeting therapies in combination with immunotherapies including immune checkpoint inhibitors.

Despite the therapeutic promise of targeting AXL in AML, several challenges remain to be addressed.

Resistance mechanisms, including acquired mutations in AXL or activation of compensatory signaling pathways, can limit the efficacy of AXL-targeted therapies, necessitating the development of next-generation inhibitors or rational drug combinations. Furthermore, the identification of predictive biomarkers to stratify AML patients who are most likely to benefit from AXL-targeted therapies is imperative for optimizing treatment selection and improving clinical outcomes. We believe that sdAbs, with their unique advantages over conventional mAbs, are promising candidates for drug development with lower immunogenicity and simplified production processes compared to conventional mAb [108].

The potential toxicity and off-target effects associated with systemic AXL inhibition underscore the need for targeted delivery strategies to minimize adverse effects while maximizing therapeutic efficacy. In conclusion, AXL represents a promising therapeutic target in AML, given its pivotal role in disease pathogenesis, adverse prognostic implications, and therapeutic vulnerabilities. Elucidating the mechanisms driving AXL dysregulation and the development of AXL-targeted therapies, especially in combinatorial regimens with chemo- and immunotherapy, hold great promise for improving the outcomes of AML patients, particularly those with high-risk disease features and refractory disease. Continued research efforts aimed at unraveling the complexities of AXL signaling and translating these findings into clinical practice are required to reveal the full therapeutic potential of AXL targeting in AML.

Abbreviations

ADC	Antibody-drug conjugates
ALK	Anaplastic lymphoma kinase
AML	Acute myeloid leukemia
BCL-2	B-cell lymphoma 2
BM	Bone marrow
BMSC	Bone marrow stromal cells
CML	Chronic myeloid leukemia
CR	Complete remission
DC	Dendritic cell
ECD	Extracellular domain
ECM	Extracellular matrix
EGF	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
ERK	Extracellular signal-regulated kinase
FDA	Food and Drug Administration
FLT3	Fms-like tyrosine kinase-3
GAS6	Growth-arrest specific protein 6
HSC	Hematopoietic stem cell
IDH	Isocitrate dehydrogenase
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ITD	Internal tandem duplication
JAK	Janus kinase
KO	Knockout
LSCs	Leukemic stem cells
MAPK	Mitogen-activated protein kinase
MCL-1	Myeloid cell leukemia-1
MDS	Myelodysplastic syndrome
MDSC	Myeloid-derived suppressor cell

MHC-I or -II	MHC class I or II
MLL	Mixed lineage leukemia
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK cell	Natural killer cell
NPM1	Nucleophosmin 1
NSCLC	Non-small cell lung carcinoma
OS	Overall survival
PDGFR	Platelet-derived growth factor receptor
PI3K	Phosphatidylinositol 3-kinase
R/R	Relapsed/refractory
RCC	Renal cell carcinoma
RON	Recepteur d'origine nantais
RTK	Receptor tyrosine kinase
STAT	Signal transducer and activator of transcription
TKI	Tyrosine kinase inhibitor
Tregs	Regulatory T cells
TGF-β	Transforming growth factor beta
TME	Tumor microenvironment
TNF	Tumor necrosis factor
VEGFR	Vascular endothelial growth factor receptor

Author contributions

N.V. and K.D.V. conceived, designed, wrote the manuscript and prepared the figures. KDV supervised the manuscript. N.D.B., A.D.B., E.D.B., E.M., K.V., K.B. and N.D. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

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Competing interests

The authors declare no competing interests.

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