Immune Evasion Mechanism and AXL

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Extensive interest in cancer immunotherapy is reported according to the clinical importance of CTLA-4 and (PD-1/PD-L1) [programmed death (PD) and programmed death-ligand (PD-L1)] in immune checkpoint therapies. AXL is a receptor tyrosine kinase expressed in different types of cancer and in relation to resistance against various anticancer therapeutics due to poor clinical prognosis. AXL and its ligand, i.e., growth arrest-specific 6 (GAS6) proteins, are expressed on many cancer cells, and the GAS6/AXL pathway is reported to promote cancer cell proliferation, survival, migration, invasion, angiogenesis, and immune evasion. AXL is an attractive and novel therapeutic target for impairing tumor progression from immune cell contracts in the tumor microenvironment. The GAS6/AXL pathway is also of interest immunologically because it targets fewer antitumor immune responses. In effect, several targeted therapies are selective and nonselective for AXL, which are in preclinical and clinical development in multiple cancer types. Therefore, this review focuses on the role of the GAS6/AXL signaling pathway in triggering the immunosuppressive tumor microenvironment as immune evasion. This includes regulating its composition and activating T-cell exclusion with the immune-suppressive activity of regulatory T cells, which is related to one of the hallmarks of cancer survival. Finally, this article discusses the GAS6/AXL signaling pathway in the context of several immune responses such as NK cell activation, apoptosis, and tumor-specific immunity, especially PD-1/PDL-1 signaling.

Keywords: PD-1/PD-L1, Gas6/Axl signaling, immune checkpoint, immune evasion, tumor microenvironment (TME), AXL

THE GAS6/AXL SIGNALING PATHWAY

Like all TYRO3, AXL, and MER (TAM) receptors, AXL is composed of two immunoglobulin-like domains, two fibronectin III (FN III) domains, a transmembrane domain, and an intracellular kinase domain (1). GAS6 is a ligand for TAM receptors, with the highest affinity for AXL (2). The gamma-carboxy glutamic (GLA) regions of GAS6 have four epithelial growth factor (EGF)-like domains and modules similar to C-terminal sex hormone-binding globulin (SHBG) that are required to activate TAM receptors. These GLA regions bind to phospholipid phosphatidylinerine (3) tethered to the extracellular surface of apoptotic cells or displayed on the outer parts of photoreceptors. Phosphatidylinerine stabilizes the interaction between TAM and its ligands such as GAS6 by increasing the binding affinity and slowing the rate of GAS6 dissociation from receptors.
GAS6 AND AXL EXPRESSION IN THE TUMOR MICROENVIRONMENT

The tumor microenvironment changes continuously during cancer progression by regulating oncogenic signals such as secreted factors and tumor-promoting cells to induce construction of tumor cells' own niche (9). While AXL expression in tumors is readily recognized, it is less well known that AXL is expressed by various cells found in the tumor microenvironment, which include several immune cell types (10), fibroblasts (11), osteoclasts (12), and endothelial cells (13–15). Furthermore, the unique tumor microenvironmental conditions may modulate AXL and GAS6 expression in both tumor and immune cells to promote aggressive and protumorigenic features. The tumor microenvironment can regulate AXL expression in various cells, and AXL seems to have a potential role in tumor development, progression, and metastasis.

AXL in Host Cells

In endothelial cells, AXL expression is involved in vascularization; i.e., when it is inhibited in tumor-bearing mice, it leads to the inhibition of tumor-induced angiogenesis (16–19). The interactions between the tumor and host immune cells in the tumor microenvironment can induce the expression of AXL and GAS6 to promote a cancerous microenvironment. Tumor cells may induce the expression of AXL and GAS6 in monocytic myeloid-derived suppressor cells (M-MDSCs) and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) (20). Moreover, generally, AXL is expressed on bone marrow-derived cells (21–24), dendritic cells (DCs) (25, 26), macrophages (27, 28), monocytes (23), natural killer (NK) cells (29), and platelets (30).

GAS6 in Host Cells

GAS6 is expressed by luminal progenitor and basal cells around the ductal lining of mammary tissue (31). In the bone microenvironment, GAS6 is secreted by osteoblasts, which are involved in bone formation (32, 33). It was demonstrated that osteoblast-derived GAS6 induces AXL expression in tumor cells (34), which suggests that paracrine GAS6/AXL signaling promotes survival, inhibits apoptosis, and mediates homing of tumor cells to the bone. In the tumor microenvironment, cancer-associated fibroblasts (CAFs) and CD45-expressing tumor-infiltrating leukocytes (TILs) express GAS6 (35–38), and CD45+ cells from the bone marrow or peripheral blood express significantly less GAS6 than TILs (38). Besides, macrophages and dendritic cells express high levels of GAS6 (37, 38), which can be further promoted by various cytokines (36). Especially in macrophages, in vitro studies suggested that tumor cells or tumor cell-conditioned media induce GAS6 expression and secretion (37, 39). Stromal cell-derived GAS6 was also shown to promote tumor cell migration, invasion, survival, and proliferation (36, 37). Potential downstream effectors of GAS6/AXL signaling through macrophage-derived GAS6 include pAKT and pSTAT3 (37).

AXL-MEDIATED TUMOR-SPECIFIC IMMUNE RESPONSE

AXL Changes Tumor Immune Microenvironment Components

During the past decades, modulating immune responses has been considered a tremendous potential therapeutics to treat cancer. Each patient’s tumor immune microenvironment (TIME) seems to be related to this treatment responsiveness. It is becoming clear that both intrinsic and extrinsic factors modulate the composition of the TIME. Specifically, several immune cells in TIME have been reported to support tumor cells’ survival through immune-suppressive functions (40, 41). Furthermore, cancer cells alter the expression of cell surface molecules to avoid detection by residential immune cells. Several studies have revealed that GAS6/AXL signaling plays a vital role in promoting the immune-suppressive tumor microenvironment. Firstly, this signaling alters the expression level of several factors, including major histocompatibility complex I (MHC-I) and programmed death ligand-1 (PD-L1) in neoplastic cells (42). However, detailed changes are different depending on cell types and research conditions. Lung adenocarcinoma cell lines (PC9 and H1975) subjected to AXL inhibition by either bemcentinib or BGB324 significantly decreased PD-L1 (42). Pharmacologic AXL inhibition using a selective AXL inhibitor (R428 or SGI-7079) in tumor cells of C57BL/6 mice significantly increased the expression of PD-1 and MHC-I molecules (43). TAM knockout mice had increased MHC-I expression of myeloid cells. In the MCF10A cells, overexpression of TAMs did not increase PD-L1 expression, but in the PD-L1–expressing MDA-MB-231 cells, treating GAS6 liposomes increased PD-L1 expression and induced AXL phosphorylation (42). Hence, further studies are warranted to understand the detailed mechanism.

Next, the GAS6/AXL signaling pathway is involved in the recruitment of both myeloid and lymphoid lineage cells, which are involved in innate and adaptive immune responses, respectively (40). It promotes the secretion of immunosuppressive cytokines, including CCL3-5, G-CSF, and TGFB (44, 45), that are involved in the infiltration of several immune cells [macrophages and myeloid-derived suppressor cells (MDSCs)], which make it possible to escape immune surveillance (42) (Figure 1). Myeloid-derived suppressor...
AXL contributes to T-cell exclusion

In addition to regulating TIME components, AXL also regulates T cell activity at different points. Usually, this helps control the excessive inflammatory response to protect normal cells; however, tumor cells take advantage of this protective mechanism by eliminating the T cell immune response toward cancer cells (51). In the study, not only the number of CD4+ and CD8+ T cells significantly increased, but also the gene expression associated with type 1 T-cell recruitment and functionality enhanced when AXL is inhibited in R428-treated tumor-bearing mice (43). Mainly, AXL receptor tyrosine kinase plays a particular role in T cell exclusion. AXL increases tumor cell invasion and metastasis by promoting T cell exclusion, acting as an inducer of tumor cell plasticity (52, 53). Genetic deletion of AXL resulted in up to 20-fold enhanced T-cell infiltration and sensitization of tumor cells to radiotherapy and checkpoint immunotherapy of a transgenic mouse model (50).

Relationship between AXL and programmed death 1

One of the immune checkpoints that are related to T cells is programmed death 1 (PD-1) and its ligand programmed cell death ligand 1 (PD-L1) (54). PD-L1 is expressed in several tumor cell types, and the interaction between PD-L1 and its receptor activates signaling pathways to prevent T-cell activation (55). Specifically, the expression of PD-L1 can serve as a dynamic mechanism for escaping host immune responses (56). For instance, neoplastic cells expressing PD-L1 have been reported to avoid cell death and continue to proliferate in the tumor microenvironment (42).

This PD-L1 immune checkpoint strongly interacts with AXL as AXL inhibition affects the PD-L1 pathway and activates the antitumor effect (43). When AXL is suppressed, the level of PD-L1 is decreased in lung adenocarcinoma and human triple-negative breast cancer cell lines (57, 58). However, tumors have a certain role regarding angiogenesis, cell invasion, metastasis, and suppression of CD8+ T cells (46, 47), and the number of these cells is reduced along with AXL knockdown (48). Lymphoid lineage cells including T cells, B-cells, and NK cells are increased when pharmacologic and genetic inhibitions of AXL are treated to cancer cells (42) (Figure 2). However, detailed parts are still to be further demonstrated because the number of tumor-infiltrating CD8+ T-cells is increased after AXL inhibition (49), while the other research showed that AXL inhibition does not affect the number of them (21, 46, 50).

Mechanisms of TAM (in particular AXL) regulation of immune evasion

- **A** Modulate TIME
  - Decrease secretion of chemokines involved in recruiting CD8+ T cells and NK cells
- **B** Promote T cell exclusion
  - Increase expression of CXC9-PD-L1
- **C** Enhance activity of T reg
  - Induce secretion of CXCL5
  - Block T cell activity
- **D** NK cell differentiation
  - Induce growth of NK cell
- **E** Suppress innate immune response
  - Inhibit AP-1
  - Block cytokine production
- **F** Inhibit apoptosis
  - Block IL-15 signaling
  - Block cell death

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** The mechanisms of TAM (in particular AXL) regulation of immune evasion. (A) Modulation of the tumor-immune microenvironment: promotes secretion of various immunosuppressive chemokine. After AXL inhibition, cytokines are decreased (CXCL9, CXCL10, CXCL11), increased (CCL-2, CCL-3, CCL-4, CCL5, CXCL1, CXCL2, CXCL5), or there could be no change (CCL12). These changes lead to regulation in the recruitment of specific immune cells (monocytes, macrophages, CD8+ T-cells, NK-cells). (B) Promoting T cell exclusion. AXL expression is significantly correlated with the expression of genes encoding CXC chemokine receptor 6 (CXCR6) and PD-L1 which prevent T cell activation. Tumors treated with the combination of pharmacological inhibition of AXL and anti-PD-1 presented an increased number of CD8 T-cells. (C) Enhancing the immune suppressive activity of regulatory T cells (Tregs). Tumor-specific Tregs can suppress antitumor immune responses in a broad range of tumor antigens, even after being activated by just one tumor-associated antigen. GAS6 induces CD4+ CD25+ Tregs to express CTLA-4 and Foxp3 especially with AXL. These activated Tregs increase the consumption of IL-2 or suppression of IL-2 production to block the activity of T lymphocytes. (D) TAM signaling is involved in the overall stage of NK cell differentiation. Especially, the GAS6/AXL pathway promotes FLT3 ligand-induced human NK cell development and cooperative interaction between the GAS6/AXL pathway and IL-15 signaling promotes NK cell differentiation. In the absence of AXL, IL-15 failed to activate several downstream signaling pathways, including PISK, AKT, and ERK1/2. (E) Suppress innate immune response. TAM signaling and type I IFN receptors inhibit the inflammatory response in macrophages and dendritic cells (DCs) by expressing the genes encoding the cytokine suppressors SOCS1 and SOCS3. In case of DCs, they serve as “presentation platforms” for GAS6 which triggers STAT1-dependent cascade with type I IFN. TAM also activates phosphorylation of DCs and macrophages to clear apoptotic cell corpses which could induce immune responses. (F) Inhibit apoptosis. The GAS6/AXL pathway is important in limiting apoptosis which involves activating survival signaling mediated by AKT, CREB, BCL-XL, and Survivin. It also suppresses phosphorylation of BAD that initiates apoptosis and activates ERK1/2 signaling.
infiltrating CD8+ T and CD4+ T cells subjected to AXL inhibition showed a noticeable induction of PD-1 on their surface. This relationship seems to be a systemic long-term memory immune response to tumor antigens (43). Furthermore, combining pharmacological inhibition of AXL with anti-PD-1 in a preclinical model of breast cancer reduces the primary tumor and metastatic burden, which is not shown when only one of them is treated. Tumors treated with the combination of these two therapeutic agents presented an increased number of CD8 T cells, with more activation of the NK cells (59). Moreover, other studies have linked AXL/PI3k signaling with increased expression of PD-L1 by tumor cells, and AXL inhibition potentiates PD-1 blockade in ID8 graft models (43, 60). This interaction is also demonstrated in lung adenocarcinoma, which showed that AXL expression significantly correlated with the expression of genes encoding PD-L1 and CXC chemokine receptor 6 (CXCR6) (57). Therefore, AXL receptor kinase is highly related to the PD-L1 immune checkpoint, contributing to immune evasion.

This relationship between AXL and PD-L1 may involve several immune cells for reactions. The analysis of TAM expression within the activated lymphoid compartment revealed that MERTK, but not AXL or TYRO3, is expressed on activated B lymphocytes and CD4+ and CD8+ T cells (61). Therefore, after apoptotic cells display PD-L1, other immune cells such as dendritic cells or macrophages sense PD-L1 involving AXL (4), and this signaling could be transferred to T-cells to take action toward immune evasion.

**REGULATORY T CELLS’ IMMUNE EVASION WITH AXL**

**Modulation of Immune Evasion With the Immune-Suppressive Activity of Regulatory T Cells**

Regulatory T cells (Tregs) mediate immune evasion, which is considered a major mechanism of escaping immune surveillance (62). Especially, tumor-derived Tregs have a relatively more effective suppressive activity than naturally occurring Tregs (63, 64). These Tregs are guided to the tumor microenvironment by tumor cell-mediated chemokine production (65, 66). After that, Tregs suppress many physical and pathological immune responses, which are crucial in sustaining self-tolerance and immune homeostasis (67).

**Contribution of Treg to Immune Suppression Through Antigen**

Naturally occurring Tregs are produced in the thymus and occupy 5%–10% of the total CD4+ T cells in the peripheral
blood, and induced Tregs derive from naive T cells under certain conditions (68). The functional importance of Tregs in a tumor-bearing host is shown in murine models of melanoma in which depleting Tregs temporarily induce an immune response against tumors and improve tumor clearance (69).

The generation and maintenance of Tregs to regulate autoimmunity require target antigens and T-cell receptor activation (70). Natural and induced Tregs independently contribute to tumor-specific tolerance. In the case of naturally occurring Tregs, an extensive unrestricted Tβ repertoire specific for a broad range of self-antigens, including tumor-associated antigens, is required which implies that the cells exercise their effect in an antigen-non-specific manner (71). One study on mice showed that induction of antigen-specific Tregs from naive cells in the tumor microenvironment does not seem to be intrinsically related to naturally occurring Tregs (72). These induced Tregs seemed to profoundly inhibit T-cell responses against tumors in an antigen-non-specific manner after being activated by a specific antigen (67). This implies that tumor-specific Tregs can suppress both medically induced and naturally occurring antitumor immune responses against a broad range of tumor antigens, even after being activated by just one tumor-associated antigen (62).

Controlling the Effect of Tregs by GAS6, Especially With the AXL Receptor

The suppressive effect of Tregs is increased by GAS6 mainly through the AXL receptor (5). Comparing how much the GAS6-induced CTLA-4 and Foxp3 expression in CD4+ CD25+ Tregs is abrogated, blocking the AXL receptor was more effective than blocking TYRO3 and MER. In addition, GAS6 has a stronger binding affinity to AXL than TYRO3 and MER (2, 73). Therefore, GAS6 has a direct role in the functions of Tregs, which enhances the suppressive activity mostly with the AXL receptor. These interactions gain credence through IL-2, a potent T cell growth factor. When CD4+CD25+T cells pretreated with GAS6 protein are cocultured with CD4+CD25+ Tregs, the proliferative activity of T cells was significantly decreased with consistent suppression of IL-2. Also, the elevated expression of CTLA-4 and Foxp3 in Tregs after GAS6 stimulation was abrogated after Axl knockdown by siRNA, and this group also showed an IL-2 level increase. These results indicate that Gas6 enhances suppression of CD4+T cells by increasing Tregs' ability to consume IL-2 or suppress IL-2 production (74).

THE ROLE OF TAM IN NK CELL ACTIVATION FOCUSING ON AXL AND IL-15

The innate immune system is usually known to recognize pathogens directly, but it also senses and destroys cells infected with pathogens. This arm of the innate response is mainly conducted by NK cells (75). Hematopoietic stem cells (HSCs) from bone marrow differentiate into common lymphoid progenitors and develop into NK cells, followed by maturation into NK cells (76, 77). Several studies have shown that NK cells are related to tumor progression in several ways, including immune evasion. Pre-metastatic niches are promoted by suppressing NK cell functions under hypoxia (78).

TAM Controls Natural-Killer-Cell Activation, Especially at the Differentiation Stage

TAM signaling plays a pivotal role in regulating the activity of NK cells (79). When NK cells are activated, they kill their targets by secreting the CD95 ligand and TNF-related apoptosis-inducing ligand (80–84), but NK cells from TAM-deficient mice have inferior cytotoxic activity (4). Mice lacking TAM possessed NK cells that have a defective function in both IFN-γ production and cytotoxicity. This activity impairment is proportional to how effectively TAM genes are inactivated as all three TAM receptors are expressed by immature NK cells in the bone marrow (79, 85). The number of NK cells generated from human CD34+ HPCs reduced after blocking GAS6 binding to AXL by AXL-Fc or warfarin (86).

After proving that TAM regulates NK cell activity, the specific stage that TAM is mainly involved in is considered. When immature cells are grown with NIH3T3 fibroblasts expressing GAS6, stromal cells can restore their ability to drive NK-cell maturation in vitro. Furthermore, the mice showed normal perforin and granzyme B levels even when NK cells lack all three TAM receptors (4). However, these cells do not secrete IFNγ, which is produced predominantly by NK cells and exhibit a 10-fold lower killing ability against target cells than wild-type NK cells after stimulation. This means that these cells do not fully demonstrate the expression of activation and inhibition receptors expressed by cytotoxic NK cells (4). Thus, TAM signaling is involved in the terminal stage of NK cell differentiation.

Importance of Interleukin-15 and AXL in NK Cell Activation

Interleukin-15 (IL-15) is another critical factor for NK cell development, which contributes to the differentiation, survival, and function of NK cells (87). When the mice are deficient in Interleukin-2 (IL-2) and IL-15 receptor, which is required only for the actions of IL-2 and IL-15 rather than other growth factors, NK cells are deficient. However, a normal level of NK cells is observed in mice deficient in IL-2 or IL-2α, which is produced predominantly by NK cells and exhibit a 10-fold lower killing ability against target cells than wild-type NK cells after stimulation. This means that these cells do not fully demonstrate the expression of activation and inhibition receptors expressed by cytotoxic NK cells (4). Thus, TAM signaling is involved in the terminal stage of NK cell differentiation.

Enhancing the Role of AXL and IL-15 Through FMS-Like Tyrosine Kinase 3

The link between IL-15 and AXL could be extended to FMS-like tyrosine kinase 3 (FLT3), one of the receptor tyrosine kinases (RTK).
Mice with genetic disruption at the FLT3 locus presented a reduction of the numbers of B-lymphoid progenitors, dendritic cells, and NK cells (92). The FLT3 ligand can enhance NK cell differentiation in the presence of IL-15 (93). To activate the FLT3 and its ligand (FL) pathway, the phosphorylation of FLT3 is essential, which follows the binding of its ligand FL (94, 95). Interruption of the GAS6/AXL pathway resulted in a marked reduction of FLT3 phosphorylation even in the presence of FL. This implies that the GAS6/AXL pathway promotes FL-induced human NK cell development by positively regulating FLT3 activation (96) (Figure 1). As FLT3 can induce differentiation of NK cells with IL-15 and the interaction between AXL and FLT3 is demonstrated, AXL and IL-15 could be highly related to each other in various immune cell activation steps.

**REGULATION OF INNATE IMMUNE CELLS BY AXL**

In addition to its role in NK cell activation, TAM has another role in regulating the innate immune response. TAM signaling is generally activated by Toll-like receptor (TLR) and type I interferon signaling, which is part of the innate inflammatory response in dendritic cells (DCs) and macrophages (3). The AXL receptor was found to be upregulated when DCs are cultured with type I IFNs (10, 26, 97). Additionally, the co-expression of TAM and type I IFN receptors in macrophages and dendritic cells (DCs) inhibits the inflammatory response of the innate immune system through induction of the genes encoding the cytokine suppressors SOCS1 and SOCS3. More specifically, the binding of the apoptotic cells to DCs is immunosuppressive and serves as "presentation platforms" for GAS6. This triggers a STAT1-dependent cascade with type I IFN, and this signaling also induces SOCS1 and SOCS3 expressions, which inhibit downstream signaling pathways of TLRs and cytokine receptors (5). Thus, the innate immune mechanism is proceeded and dependent on TAM receptors.

**REGULATION OF IMMUNE EVASION BY AXL THROUGH APOPTOSIS AND PHAGOCYTOSIS**

Several pieces of research have shown the relationship between AXL and apoptosis. Apoptosis produces materials that can induce an immune response, which should be prevented for tumor immune evasion.

**Inhibition of Apoptosis by GAS6 With AXL Kinase**

Apoptosis of vascular smooth muscle cells (VSMCs) has been identified in the physiological remodeling of the vasculature. Cell death with cell proliferation, migration, and matrix turnover may induce changes in vascular architecture during development and diseases such as atherosclerosis (98). This apoptosis of VSMCs is coupled with GAS6 binding to the AXL receptor, and GAS6 inhibits apoptosis in cultured VSMCs through AXL phosphorylation (99). GAS6 and AXL are increased after vascular injury, and these molecules play an essential role in neointima formation by suppressing apoptosis (100). Besides, it is speculated that the GAS6/AXL pathway is vital to limiting VSMC apoptosis by activating AKT and PI3K along with several other pathways, including phosphorylation of BAD (BCL2-associated agonist of cell death) and activation of ERK1/2 (101–103).

Non-small cell lung cancer (NSCLC), a prevalent and devastating disease, shows overexpression and activation of MER and AXL. MER or AXL knockdown also improved *in vitro* NSCLC sensitivity to chemotherapeutic agents by promoting apoptosis. Also, AXL inhibition induces apoptosis by abating survival signaling mediated by AKT, CREB, BCL-XL, and survivin (104).

**Removal of Apoptosis Remains by Activating Phagocytosis**

Furthermore, some cancers develop specific mechanisms to clear apoptotic cells to regulate immune responses. Defects in the clearance of apoptotic cells can induce an immune response. Macrophages and DCs must remove many apoptotic cells corpses, but this form of homeostatic phagocytosis is impaired in TAM-deficient mice (24, 105, 106). This immunosuppressive effect of DCs is exerted through TAM signaling, and this immune response may be reinforced, especially in cancer cells as they overexpress AXL. Hence, TAM contributes to immune evasion by activating phagocytosis to remove these remains.

However, there is a possibility that TAM signaling induced by apoptotic cells is autocrine which means macrophages and DCs themselves produce GAS6, not from the apoptotic cells. In this case, phosphatidylserine is the primary stimulant that stabilized the interaction between TAM and its ligands (4). Thus, further research is needed to understand the exact mechanism.

**CLINICAL TRIAL SUPPRESSING AXL**

Targeting AXL for cancer treatment is under the spotlight, and several clinical studies involving the use of anti-AXL have been conducted. The first clinical trial treating an anti-AXL-specific small molecule inhibitor called BGB324 was performed in 2013. BGB324 blocks auto-phosphorylation of AXL on the COOH-terminal multiple docking sites Tyr821 with the subsequent activation of AKT and SFK phosphorylation (107). After entering phase I clinical trials in 2013, it is currently under phase II study to assess the safety of BGB324 when given in up to 77 patients advanced adenocarcinoma of the lung previously treated with pembrolizumab (108). Since then, several newly synthesized inhibitors specific for AXL receptors are being tested at a clinical stage.

Many small-molecule inhibitors in the clinical stage do not solely target AXL. Some inhibitors work as AXL pathway modulators that target factors such as MET, TYRO3, and FLT3 along with AXL and are used with several immune checkpoints inhibitors (ICIs), including nivolumab, pembrolizumab, durvalumab, and avelumab (109).

Small molecules are being developed mainly, and other therapeutic agents such as a monoclonal antibody or nucleotide
aptamer are also in preclinical progress. The YW327.6S2 phage-derived monoclonal antibody binds to human AXL with high affinity, which blocks the GAS6 binding to the receptor and downregulates receptor expression (110). Aptamers are short structured single-stranded RNAs or DNAs that bind to a specific target molecule. They are promising alternatives with great potentials because of their low cost, lower toxicity, and higher affinity (111, 112). A selective RNA-based aptamer, GL21.T, binds the extracellular domain of AXL at high affinity and inhibits its catalytic activity. This includes ERK and AKT phosphorylation and inhibited in vivo lung tumor formation in mouse xenografts (111) (Table 1).

### AUTHOR CONTRIBUTIONS

H-YS contributed to the conception and design of the study. H-YS and H-KJ wrote sections of the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

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