

Keywords: AXL; TYRO3; AXL; MERTK (TAM) family; receptor tyrosine kinase; chemoresistance; immune checkpoint blockade; epithelial to mesenchymal transition

Giving AXL the axe: targeting AXL in human malignancy

Carl M Gay¹, Kavitha Balaji¹ and Lauren Averett Byers^{*,1}

¹Department of Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA

The receptor tyrosine kinase AXL, activated by a complex interaction between its ligand growth arrest-specific protein 6 and phosphatidylserine, regulates various vital cellular processes, including proliferation, survival, motility, and immunologic response. Although not implicated as an oncogenic driver itself, AXL, a member of the TYRO3, AXL, and MERTK family of receptor tyrosine kinases, is overexpressed in several haematologic and solid malignancies, including acute myeloid leukaemia, non-small cell lung cancer, gastric and colorectal adenocarcinomas, and breast and prostate cancers. In the context of malignancy, evidence suggests that AXL overexpression drives wide-ranging processes, including epithelial to mesenchymal transition, tumour angiogenesis, resistance to chemotherapeutic and targeted agents, and decreased antitumor immune response. As a result, AXL is an attractive candidate not only as a prognostic biomarker in malignancy but also as a target for anticancer therapies. Several AXL inhibitors are currently in preclinical and clinical development. This article reviews the structure, regulation, and function of AXL; the role of AXL in the tumour microenvironment; the development of AXL as a therapeutic target; and areas of ongoing and future investigation.

AXL, first isolated in 1988 in a screen for transforming genes in patients with chronic myeloid leukaemia that progressed to 'blast crisis' (Liu *et al*, 1988), was later characterised by two groups in 1991 (Janssen *et al*, 1991; O'Bryan *et al*, 1991). O'Bryan *et al* identified an overexpressed, transforming complementary DNA (cDNA) in human myeloid leukaemia cells that they called AXL, a name derived from the Greek anexelekto, meaning uncontrolled. Simultaneously, Janssen *et al* independently identified the same transforming cDNA, which they called UFO (a reference to its unidentified function), from NIH3T3 mouse fibroblasts transfected with DNA from a patient with a chronic myeloproliferative disorder. These cDNAs were predicted to encode a novel receptor tyrosine kinase (RTK), now referred to as AXL or UFO. In the years since its identification, AXL has become an increasingly attractive target for anticancer therapies given its implication in an ever-expanding list of cellular processes across various normal and malignant tissue types.

STRUCTURE, SIGNALLING, AND REGULATION

The AXL protein is characterised by an extracellular structure consisting of two fibronectin type 3-like repeats and two immunoglobulin-like repeats along with its intracellular tyrosine kinase domain. Along with the other members of the TYRO3, AXL, and MERTK

(TAM) family, AXL has a KWIAIES amino acid sequence within its intracellular tyrosine kinase domain (Graham *et al*, 2014). The KWIAIES motif, unique to TAM family members, is critical for kinase activity and shares close homology to similar sequences in related tyrosine kinases, including a similar motif in RET, which harbours the M918T-activating mutation frequently found in medullary thyroid cancer (Toledo *et al*, 2016). AXL also broadly shares homology with other RTKs, including FGFR, EGFR, and PDGFR.

AXL, like the other TAM members, is activated in part via interaction with the vitamin K-dependent protein ligand growth arrest-specific protein 6 (GAS6; Stitt *et al*, 1995; Varnum *et al*, 1995). Studies suggest that this interaction may be constitutive and is not sufficient for activation of downstream effectors (Fujimori *et al*, 2015). Additional evidence points to GAS6-independent mechanisms of AXL activation. In the context of AXL overexpression, abundant AXL protein may lead to aggregation of AXL extracellular domains on adjacent cells (Bellosta *et al*, 1995) or even ligand-independent homodimerisation (Burchert *et al*, 1998) with subsequent downstream activation in both cases. In addition, AXL may heterodimerise with non-TAM RTKs and initiate AXL-dependent programs with or without their dimerisation partner's ligand (Meyer *et al*, 2013; Vouri *et al*, 2016). Canonical activation of AXL via GAS6 also requires an additional interaction between GAS6 and the phospholipid phosphatidylserine (PtdSer; Meyer

*Correspondence: Dr LA Byers; E-mail: lbyers@mdanderson.org

Received 27 September 2016; revised 21 November 2016; accepted 23 November 2016; published online 10 January 2017



et al, 2015). Although ubiquitously expressed in cell membranes, PtdSer normally is located exclusively in the inner portion of the phospholipid bilayer. However, upon apoptotic cell death, PtdSer flips to the external portion of the bilayer, where it is accessible to the AXL–GAS6 complex on adjacent cells (Ravichandran, 2010).

Following activation, AXL undergoes homodimerisation, autophosphorylates and transphosphorylates its intracellular tyrosine residues, and recruits SH2 domain-containing effector molecules and adaptor proteins to these phosphotyrosine residues (Braunger *et al*, 1997; Sasaki *et al*, 2006). Signalling pathways activated downstream of AXL (Figure 1) include PI3K–AKT–mTOR, MEK–ERK, NF- κ B, and JAK/STAT (Fridell *et al*, 1996; Tai *et al*, 2008; Ruan and Kazlauskas, 2012; Pacciez *et al*, 2013). The function of activated AXL in normal tissues includes the efficient clearance of apoptotic material and the dampening of TLR-dependent inflammatory responses and natural killer cell activity (Sharif *et al*, 2007; Rothlin *et al*, 2007). AXL loss-of-function results in increased

inflammation and even autoimmunity (Weinger *et al*, 2011; Nguyen *et al*, 2013; Li *et al*, 2015).

AXL's role in reducing inflammation may also be exploited by viruses that evade immune response by externalising PtdSer, thereby activating TAM RTKs and surreptitiously gaining entry into cells. Specifically, AXL has been proposed as a putative entry receptor for West Nile, Ebola, and Zika viral infections (Lantin le Boulch *et al*, 1991; Perera-Lecoin *et al*, 2014; Nowakowski *et al*, 2016). Further illustrating this role, AXL is upregulated *in vivo* after hepatitis C virus infection, and *in vitro* findings suggest that AXL inhibits interferon alpha and, therefore, antiviral response (Read *et al*, 2015).

AXL protein is expressed in normal tissues, particularly in bone marrow stroma and myeloid cells, and in tumour cells and tumour vasculature (Neubauer *et al*, 1994; Shieh *et al*, 2005). AXL expression in these tissues raises concern for potential haematologic and/or immune side effects, including autoimmunity and

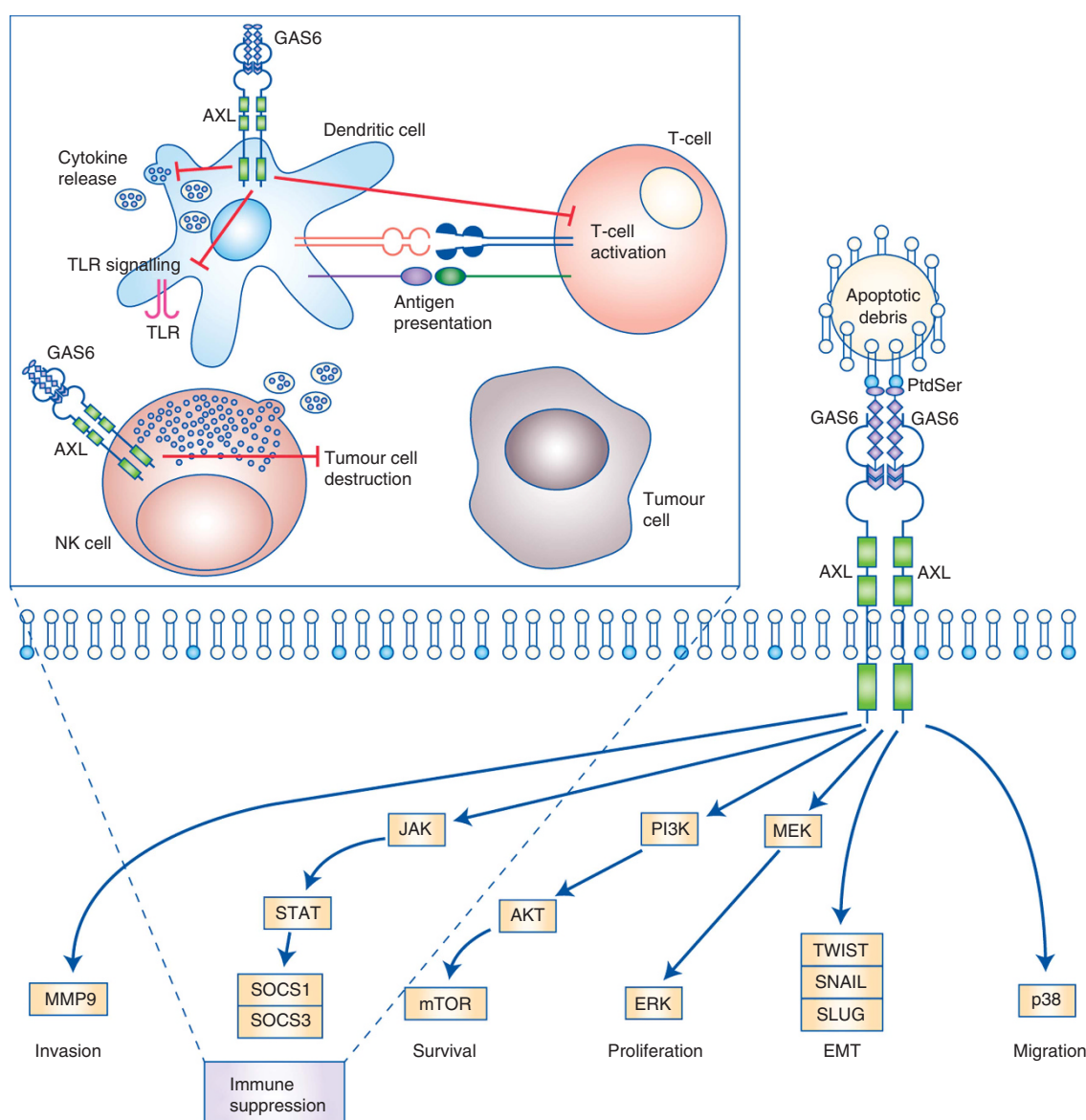


Figure 1. Spectrum of cellular processes regulated by AXL activity. AXL, following activation by its ligand GAS6 along with an interaction between GAS6 and phosphatidylserine (PtdSer), dimerises and cross-phosphorylates (yellow circle) its partner receptor. This activation regulates an array of cellular pathways as illustrated at the bottom of the figure. Inset: AXL activity plays a complex role in immune regulation that includes the inhibition of cytokine release, TLR signalling, and T-cell activation by antigen-presenting cells such as dendritic cells (above), as well as specific antitumor killing by natural killer cells (below).

even inflammation-induced malignancy, associated with AXL blockade (Bosurgi *et al*, 2013).

The regulation of AXL/AXL expression includes both transcriptional and post-transcriptional mechanisms (Figure 2A). MZF1 has been shown to bind to the AXL promoter, to transactivate promoter activity, and, in turn, to result in a dose-dependent increase in AXL mRNA expression (Mudduluru *et al*, 2010b). Additional transcription factors have been implicated less definitively in AXL regulation, including HIF1 α and AP1 (Mudduluru *et al*, 2010a; Rankin *et al*, 2014). Other data indicate that AXL expression is further regulated by methylation of CpG islands, which flank Sp1 transcription factor binding sites in the AXL promoter (Mudduluru and Allgayer, 2008). Analyses in dendritic cells showed that AXL mRNA is upregulated in a STAT1-dependent manner downstream of activation of TLR signalling (Rothlin *et al*, 2007). Similarly, in macrophages, AXL expression is induced by TLR ligands and other markers of inflammation, including tumour necrosis factor, but this effect was inhibited by treatment with corticosteroids (Zagorska *et al*, 2014).

In dendritic cells, AXL expression is abundant, and in bone marrow-derived macrophages, AXL expression is minimal; however, there is essentially no difference in AXL mRNA copy number in these cells, suggesting a significant role for post-transcriptional regulation of AXL expression (Zagorska *et al*, 2014). Supporting the role of post-transcriptional AXL regulation, one study found that AXL is a target for the ubiquitination activity of the E3 ligase Cbl-B in natural killer cells (Figure 2B; Paolino *et al*, 2014). Other studies have identified target sequences for microRNA (miRs)

including miR-34 and miR-199a/b in the AXL 3' untranslated region (Figure 2C), with correlative findings confirming the effects of miRs on AXL expression (Mackiewicz *et al*, 2011; Mudduluru *et al*, 2011). Further supporting the role for post-transcriptional regulation of AXL expression is the fact that, in spite of frequent AXL overexpression in many tumour types, genetic mutation and amplification events are relatively rare in these malignancies. AXL mutations, fusions and/or amplifications are found in 3% or fewer of breast cancer, head and neck squamous cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma and acute myeloid leukaemia (Figure 2D) – each an example of a malignancy in which AXL over-expression is proposed to play a significant role in disease development, progression, metastasis or treatment resistance (Cancer Genome Atlas Research N, 2012; Cancer Genome Atlas Research N, 2013; Cancer Genome Atlas Research N, 2014; Cancer Genome Atlas N, 2015; Ciriello *et al*, 2015). One study reported a fusion gene construct between AXL and MBIP in large-scale sequencing of primary lung adenocarcinoma samples (Seo *et al*, 2012), but this fusion event has not been reported elsewhere and is unlikely to offer further insights into the overexpression of AXL seen in these tumour types. Contrastingly, mRNA and protein expression analyses suggest high AXL/AXL expression in 32% and 33–48%, respectively, of lung adenocarcinoma samples (Shieh *et al*, 2005; Ishikawa *et al*, 2013). Similar discordance is noted between genetic alterations and mRNA/protein expression in head and neck squamous cell carcinoma (Lee *et al*, 2012), and acute myeloid leukaemia (Ben-Batalla *et al*, 2013).

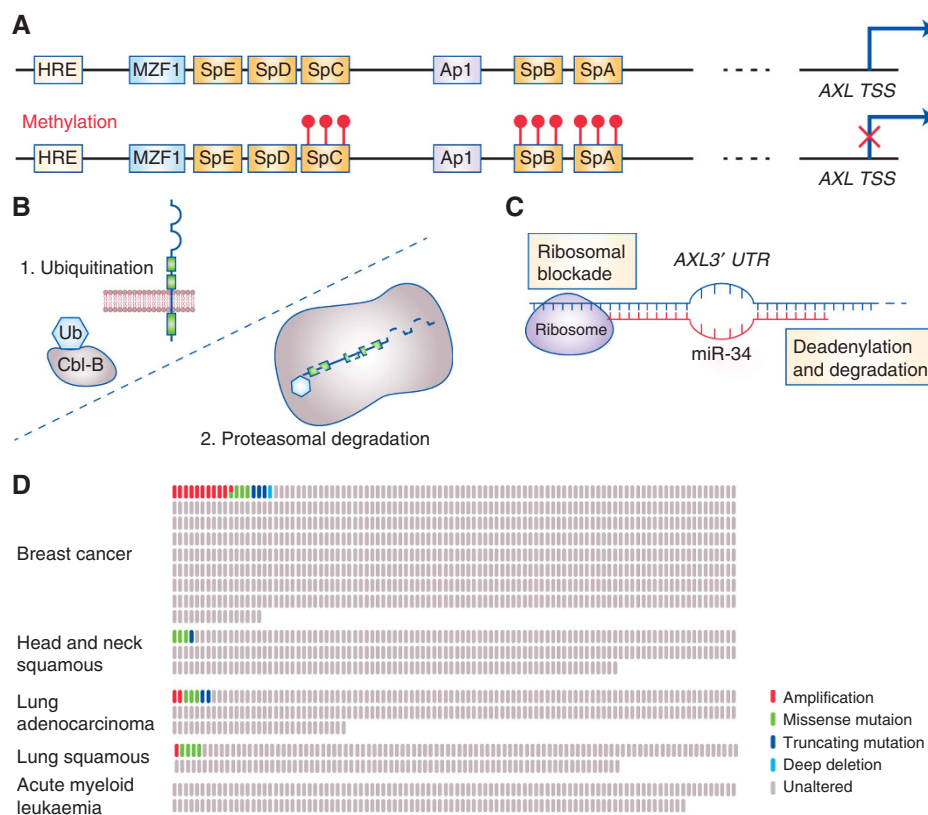


Figure 2. Transcriptional and post-transcriptional regulation of AXL/AXL. (A) The promoter region upstream of the AXL transcriptional start site (TSS) highlights binding sites for transcription factors, including hypoxia responsive element (HRE) for HIF1 α , MZF1, and AP1. The promoter region also contains numerous Sp1 binding sites, which are sites of potential repressive methylation events (red). (B) AXL is a target for Cbl-B-dependent ubiquitination (Ub) resulting in proteasomal degradation. (C) AXL 3' untranslated region (UTR) contains target sequences for miRs, including miR-34 (pictured) and miR-199a/b, resulting in reduced translation via ribosomal blockade and increased RNA degradation. (D) Data adapted from cBioPortal show the infrequency of AXL genetic alterations in selected tumour types. Frequencies are as follows: breast cancer, 2.2% (18/816); head and neck squamous cell carcinoma, 1.4% (4/279); lung adenocarcinoma, 3% (7/230); lung squamous cell carcinoma, 2.8% (5/178); and acute myeloid leukaemia, 0% (0/191).

AXL EXPRESSION IN THE TUMOUR AND TUMOUR MICROENVIRONMENT

AXL is a putative driver of diverse cellular processes that are critical for the development, growth, and spread of tumours, including proliferation, invasiveness and migration, EMT, stemness, angiogenesis, and immune modulation.

AXL activity and its inhibition have been demonstrated to modulate proliferation in various tissue and tumour types via diverse downstream effectors, including AKT, MAPK, and NF- κ B. Small hairpin RNA-mediated knockdown of AXL expression in osteosarcoma cells has been associated with decreased proliferation, as marked by Ki-67 expression, and increased expression of apoptotic markers (Zhang *et al*, 2013). Similarly, in prostate cancer cell lines, treatment with GAS6 stimulated proliferation (Sainaghi *et al*, 2005), whereas AXL knockdown predictably led to decreased proliferation (Paccez *et al*, 2013). Additional data suggest a similar role for AXL in stimulating proliferation in mesothelioma, lung adenocarcinoma, colorectal adenocarcinoma, and other malignancies (Ou *et al*, 2011; Cui *et al*, 2012; Yuen *et al*, 2013).

Both *in vitro* and *in vivo* data implicate AXL as a driver of invasiveness and migration. Small hairpin RNA knockdown of AXL resulted in decreased migration and invasion in colorectal and cervical cancer cell lines (Mudduluru *et al*, 2010b). AXL activity correlates with a migratory cellular phenotype, including increased GTP-bound forms of Rho and Rac (Koorstra *et al*, 2009) and filopodial formation (Lay *et al*, 2007). In osteosarcoma cell lines, AXL activation correlated with phosphorylated AKT and MMP9 expression and promoted cell migration and invasion *in vitro* (Han *et al*, 2013). In fact, MMP9 dependence is a recurring feature in multiple studies that highlight the role of AXL in stimulating migration and invasion. For example, inflammatory breast cancer cell lines depleted of TIG1, a protein predicted to stabilise and prevent the degradation of AXL, have decreased MMP9 expression and decreased *in vitro* invasion and migration (Wang *et al*, 2013). MMP9 expression is also enhanced *in vivo* by AXL in an NF- κ B-dependent manner (Tai *et al*, 2008).

In light of AXL's role in invasiveness and migration, it is unsurprising that multiple studies have found an association between AXL and EMT. AXL was strongly correlated with a mesenchymal phenotype in a 76-gene EMT signature in NSCLC (Byers *et al*, 2013), and this correlation subsequently has been substantiated in additional tumour types (Mak *et al*, 2016). Our data and other studies have shown that AXL knockdown leads to downregulation of transcription factors required for EMT, including *Slug*, *Twist*, and *Zeb1*, and to increased expression of E-cadherin (Asiedu *et al*, 2014; Lee *et al*, 2014; unpublished data). Furthermore, depletion of AXL in squamous cell carcinoma cell lines increases cell-cell adhesion, suggesting a reversion to an epithelial-type morphology (Cichon *et al*, 2014).

AXL plays an important role in stem cell maintenance. For example, AXL expression is positively regulated by EZH2 in glioma cells, and silencing AXL in these cell lines mimicked the effect of EZH2 inhibition (Ott *et al*, 2012). EZH2 has been suggested to play a crucial role in stem cell maintenance. Supporting AXL's function in stem cell maintenance, one study of cutaneous squamous cell carcinoma cell lines showed that downregulation of AXL correlated with loss of cell-cell adhesion and diminished TGF β -R and WNT signalling, while AXL activity correlated with expression of stem cell markers such as CD44 and ALDH1 (Cichon *et al*, 2014). Similarly, in murine breast cancer cell lines, AXL expression correlated with expression of stem cell markers, whereas downregulation of AXL resulted in loss of the capacity for self-renewal (Asiedu *et al*, 2014).

AXL normally is expressed in capillary endothelium and vascular smooth muscle cells (O'Donnell *et al*, 1999), and several

lines of evidence support a role for AXL in promoting angiogenesis. For example, *Axl*-null mice have a deficient angiogenic and vascular permeability response to VEGF-A (Ruan & Kazlauskas, 2012). Findings from human endothelial cells suggest that lactate-dependent activation of AXL, along with TIE2 and VEGFR2, promotes PI3K/AKT activity and subsequent angiogenesis (Ruan & Kazlauskas, 2013). The AXL ligand GAS6 also has been proposed as a chemoattractant capable of inducing AXL-mediated migration of vascular smooth muscle cells (Fridell *et al*, 1998). Furthermore, HIF1 α , a major mediator of hypoxia-induced genetic programs known to promote angiogenesis, has a direct binding site in the AXL promoter (Rankin *et al*, 2014).

However, data from other experimental models suggest a potential antiangiogenic role for AXL. In chick chorion allantoic membrane samples, GAS6 appears to inhibit VEGFA:VEGFR2-dependent angiogenesis in an AXL-dependent manner (Gallicchio *et al*, 2005). These data highlight a complex role for AXL signalling in regulating both normal and tumour vasculature.

Given the recent emphasis on the role of immune evasion in tumour development and metastasis, it is not surprising that the well-characterised role of AXL in suppressing inflammation and autoimmunity has emerged as a focal point in AXL research. One study supported the capability of TAM RTKs to hijack proinflammatory signals to activate suppressors of cytokine and TLR signalling, serving as a *de facto* feedback mechanism to prevent autoimmunity (Rothlin *et al*, 2007). Several experiments have illustrated the potential effect of AXL signalling on immune surveillance for tumour cells. One study found that treatment of murine melanoma or breast cancer models with a TAM inhibitor reduced AXL activity and markedly decreased metastases; the authors suggested that AXL (along with other TAMs) decreased the activity of natural killer cells and their ability to eliminate metastases (Paolino *et al*, 2014). Similarly, findings from murine breast cancer xenograft models showed that treatment with a monoclonal antibody that binds both human and murine AXL inhibited activity of tumour-associated macrophages (Ye *et al*, 2010). These data support the idea that increased AXL expression may be associated with decreased immune response to tumour cells. Other data posit a role for AXL's immunomodulatory function in tumour development. For example, loss of AXL and *Mertk* signalling in normal tissue was associated with increased susceptibility to the induction of inflammatory signalling and, ultimately, inflammation-induced malignancy in mice (Bosurgi *et al*, 2013).

AXL AS DRIVER OF THERAPEUTIC RESISTANCE

AXL has been suggested to promote both intrinsic and acquired resistance to chemotherapeutic, immunotherapeutic and molecularly targeted agents in both solid and haematologic malignancies. In the earliest example of AXL-associated drug resistance, the expression of AXL mRNA in cisplatin-resistant ovarian cancer cells was double that in sensitive cells (Macleod *et al*, 2005). Similarly, AXL expression is induced by chemotherapy treatment and has been correlated with Bcl2 and Twist expression and with chemoresistance in acute myeloid leukaemia cell lines (Hong *et al*, 2008). Additional studies have shown comparable correlations between AXL expression and chemoresistance in breast, colon, and lung cancers and in other cancers (Asiedu *et al*, 2014; Heckmann *et al*, 2014; Kim *et al*, 2015).

There are myriad examples of AXL activity correlating with resistance to targeted therapies. For example, in EGFR-mutated NSCLC, AXL was the most highly overexpressed gene in erlotinib-resistant xenograft models, and treatment with an AXL inhibitor restored sensitivity to erlotinib in an otherwise resistant cell line

(Zhang *et al*, 2012). Further findings showed that the overexpression of a wild-type AXL construct was sufficient to impair response to erlotinib *in vitro*, whereas a kinase-impaired AXL mutant induced no such resistance (Zhang *et al*, 2012). Upregulation of AXL protein was seen in 7 of 35 patient samples of EGFR-mutated NSCLC taken before treatment with EGFR inhibitor and after resistance occurred, including 2 of 8 samples with the EGFR p.Thr790Met resistance mutation (Zhang *et al*, 2012). Comparable data from renal cell carcinoma xenograft models show upregulation of AXL and MET in resistance to long-term sunitinib therapy, as well as resensitisation to sunitinib after AXL and MET inhibition via treatment with the tyrosine kinase inhibitor cabozantinib (Zhou *et al*, 2016). Prominent upregulation of AXL was also found in gastrointestinal stromal tumours with resistance to imatinib (Mahadevan *et al*, 2007). Selective knockdown of AXL restored sensitivity in imatinib-resistant chronic myeloid leukaemia cell lines (Dufies *et al*, 2011). Additional evidence supports a correlation between AXL expression and resistance to HER2 inhibitors in HER2-amplified breast cancer (Liu *et al*, 2009) and oesophageal squamous cell carcinoma (Hsieh *et al*, 2016), as well as resistance to cetuximab in head and neck squamous cell carcinoma (Brand *et al*, 2014, 2015).

Many studies have also linked AXL expression to resistance to targeted therapies beyond RTK targeted agents. For example, in *PIK3CA* mutant or amplified head and neck squamous cell carcinoma, resistance to PI3K inhibitors is linked to high AXL expression (Elkabets *et al*, 2015). Mechanistic studies have suggested that PI3K-independent activation of mTOR occurs as a result of AXL-EGFR dimerisation and activation of PLC γ (Elkabets *et al*, 2015). Similarly, in BRAF V600E mutant melanoma, low MITF to AXL expression ratio is associated with resistance to BRAF inhibitors (Konieczkowski *et al*, 2014; Muller *et al*, 2014). Recent analyses investigated AXL and MITF expression with single cell resolution in melanoma and found that every tumour analysed possessed both AXL-high and MITF-high cells at baseline (Tirosh *et al*, 2016). However, there was a statistically significant tendency for tumours to show predominant AXL-high transcriptomic programs upon relapse following BRAF \pm MEK inhibition compared with matched pretreatment samples (Tirosh *et al*, 2016). These data suggest that small populations of AXL-high cells are preexistent within the tumour and that their persistence and/or proliferation may drive inherent resistance even in an apparently responding tumour leading to inevitable relapse. This fits clinical observations wherein appropriately targeted therapies almost invariably generate an initial clinical response that is, unfortunately, short-lived because of almost-as-inevitable therapeutic resistance.

The increasing role of immune checkpoint blockade has led to rapidly growing interest in resistance mechanisms to these agents and, perhaps expectedly given AXL's role in immune evasion, emerging data show that increased AXL expression is a component of an anti-PD-1 resistance program in non-responders (Hugo *et al*, 2016). Even radiation resistance has been linked to AXL expression, as radiation-resistant HNSCC xenograft and patient-derived xenograft models expressed increased AXL, whereas AXL knockdown restored sensitivity to radiation in HNSCC cell lines (Brand *et al*, 2015).

Unsurprisingly, AXL/AXL expression, or in some cases overexpression, correlates with poor prognosis in multiple tumour types including lung adenocarcinoma (Ishikawa *et al*, 2013), breast invasive ductal carcinoma (Tanaka *et al*, 2016), high-grade ovarian cancers (Lozneau *et al*, 2016), oesophageal squamous cell carcinoma (Hsieh *et al*, 2016). The prognostic implication of *GAS6*/*GAS6* expression is less clear, with data supporting *GAS6* protein as a poor prognostic marker in lung

adenocarcinoma (Ishikawa *et al*, 2013), but data finding either no significance (Ben-Batalla *et al*, 2013) or better prognosis (Ishikawa *et al*, 2013) with increased *GAS6* mRNA expression in acute myeloid leukaemia and lung adenocarcinoma, respectively. This raises the possibility that *GAS6*-independent activation of AXL, as previously described, may be driving therapeutic resistance and prognosis.

AXL AS THERAPEUTIC TARGET

Because of AXL's crucial role in both tumour biology and therapeutic resistance, AXL is an attractive target for antineoplastic therapies. Recent preclinical studies have shown benefits of AXL inhibition in such diverse scenarios as increasing apoptosis in glioblastoma (Onken *et al*, 2016), sensitising tumours to PARP inhibition (Balaji *et al*, 2017), overcoming resistance to PI3K inhibitors (Elkabets *et al*, 2015) and synergising with anthracycline treatment in breast cancer models (Wang *et al*, 2016), among others. Several targeted therapies in development and already in use have nonspecific activity against AXL (Table 1), including bosutinib, approved by the United States Food and Drug Administration for Philadelphia chromosome-positive chronic myeloid leukaemia. Bosutinib targets SRC/ABL tyrosine kinases in addition to AXL and can overcome resistance to imatinib, which may be AXL-dependent (Khouri *et al*, 2012). A similar ability to overcome imatinib resistance in gastrointestinal stromal tumour cell lines has been shown with amuvatinib, an inhibitor of c-Kit, FLT3, RET, PDGFR β , and AXL (Mahadevan *et al*, 2015).

Cabozantinib, another multi-kinase inhibitor, targets VEGFR, MET, FLT3, c-Kit, and AXL and has been approved by the United States Food and Drug Administration for treating both medullary thyroid cancer and renal cell carcinoma. Several ongoing clinical trials (Table 1) are investigating cabozantinib as a treatment for NSCLC (as a monotherapy: NCT01639508; in combination with erlotinib: NCT00596648, NCT01708954, and NCT01866410) and may highlight its activity against AXL in overcoming or delaying resistance to EGFR inhibitors. A similar trial is investigating cabozantinib in combination with panitumumab, an EGFR-targeting monoclonal antibody, in KRAS wild-type colorectal cancer (NCT02008383).

MET/AXL inhibitor glesatinib (MGCD265) yielded a striking clinical response when used to treat a patient with metastatic NSCLC with AXL amplification (Do *et al*, 2015). However, AXL amplification was identified in only 0.7% of the 408 NSCLC samples analysed as part of the lung adenocarcinoma and lung squamous cell carcinoma TCGA projects (Cancer Genome Atlas Research N, 2012; Cancer Genome Atlas Research N, 2014), suggesting such an amplification event is rare. This drug is currently in an ongoing Phase 2 trial (NCT02544633) for patients with NSCLC expressing MET alterations.

Several specific AXL inhibitors have recently entered early-phase clinical trials, including BGB324 (previously R428; in combination with erlotinib in NSCLC: NCT02424617; in combination with cytarabine in acute myeloid leukaemia: NCT02488408) and BPI-9016M (safety in advanced solid tumours: NCT02478866). A monoclonal antibody targeting AXL (YW327.6S2) and an AXL decoy receptor (GL2L.T) are currently in preclinical development. Additionally, an oral AXL inhibitor (TP-0903) is expected to enter Phase 1 clinical trial in November 2016 (in advanced solid tumours: NCT02729298). These approved drugs and ongoing and pending clinical trials highlight the potentially wide-ranging safety and efficacy of AXL inhibition.

Table 1. AXL-targeting drugs in various phases of development

Drug	Target(s)	AXL IC50	Phase of approval	Indication
BGB324	Axl	14 nM	Phase I/II NCT02424617	NSCLC (+ erlotinib)
BGB324	AXL	14 nM	Phase I NCT02488408	AML (± cytarabine)
Bosutinib	SRC/ABL, AXL	174 nM	Approved	Ph (+) CML
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Approved	medullary thyroid cancer, renal cell carcinoma
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Phase II NCT01639508	NSCLC with RET, ROS1 and NTRK fusions or increased MET or AXL activity
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Phase II NCT01708954	NSCLC (± erlotinib)
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Phase II NCT01866410	NSCLC (+ erlotinib)
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Phase I NCT02008383	KRAS WT CRC (+ panitumumab)
Cabozantinib	VEGFR, MET, FLT3, c-KIT, AXL	7 nM	Phase I NCT00596648	NSCLC (± erlotinib)
MGCD265	MET, AXL	1 nM ≤ IC50 ≤ 10 nM	Phase I NCT00697632	Advanced solid tumours
ASLAN002	RON, AURKA, FLT3, MET, AXL	1.1 nM	Phase I NCT01721148	Advanced solid tumours
MGCD516	MET, MER, VEGFR, PDGFR, DDR2, TRK, EPH, AXL	11 nM	Phase I NCT02219711	Advanced solid tumours

AXL IN THE FUTURE

Based on our current knowledge of AXL's role in therapy resistance, future studies will help to determine whether AXL has a translational application as a biomarker for predicting therapeutic response to established drugs. Beyond this, the growing number of AXL inhibitors, and the ongoing clinical trials employing them, will allow us to determine the therapeutic potential of AXL targeting. However, unlike other targeted agents such as EGFR or ALK inhibitors, alterations at the DNA level are uncommon and are unlikely to be the optimal biomarkers for AXL inhibitors. It will be important to explore in clinical trials whether AXL expression levels (either protein or mRNA) can be used to identify those patients who get the most benefit from AXL targeting and, if so, what the most robust assays will be for quantifying this biomarker. AXL may also prove important in the field of cancer immunotherapy. Currently, even among those malignancies for which they are approved, the majority of patients do not respond to immune checkpoint blockade and even more develop resistance to these drugs after initial response. In light of AXL's role in suppressing immune response, there is a strong rationale for pursuing AXL inhibition in combination with immune checkpoint blockade in a clinical trial setting to overcome this resistance and enhance antitumor immunity. Given AXL's putative role as a mediator of EMT and cancer stemness, inhibiting AXL may also reveal intriguing results regarding the role of these processes in metastatic potential and/or chemosensitivity and chemoresistance. In particular, clinical trials expanding on the preclinical data suggesting that inhibition of AXL could reverse resistance to conventional chemotherapies or targeted therapies, are already being pursued with more likely to follow. Further research is also needed to elucidate the precise downstream signalling mechanisms required for each of AXL's roles to design rational combination therapies and to determine mechanisms of resistance. AXL has emerged as a major therapeutic target and a potential biomarker in several cancer types, and future

investigations are warranted to develop novel and effective treatment and diagnostic tools based on this target.

ACKNOWLEDGEMENTS

LAB is supported, in part, by the R Lee Clark Fellow Award (supported by the Jeane F. Shelby Scholarship Fund); the MDACC Physician Scientist Award; the LUNgevity Foundation; the University of Texas-Southwestern and MD Anderson Cancer Center Lung SPORE (5 P50 CA070907); the NCI Cancer Clinical Investigator Team Leadership Award (P30 CA016672); and an NIH/NCI R01 award (1 R01 CA207295-01). We also thank Jill Delsigne-Russell for her editorial contributions to the manuscript.

CONFLICT OF INTEREST

CMG and KB report no disclosures or conflicts of interest. LAB reports personal fees from Medivation, Inc., personal fees from AstraZeneca Pharmaceuticals, personal fees from AbbVie, personal fees from BioMarin Pharmaceutical Inc., and personal fees from Astex Therapeutics outside the submitted work. She is also site principal investigator for the referenced clinical trial NCT02424617.

REFERENCES

- Asiedu MK, Beauchamp-Perez FD, Ingle JN, Behrens MD, Radisky DC, Knutson KL (2014) AXL induces epithelial-to-mesenchymal transition and regulates the function of breast cancer stem cells. *Oncogene* **33**(10): 1316–1324.
- Balaji K, Vijayaraghavan S, Diao L, Tong P, Fan Y, Carey JP, Bui TN, Warner S, Heymach JV, Hunt KK, Wang J, Byers LA, Keyomarsi K (2017) AXL inhibition suppresses the DNA damage response and sensitizes cells to PARP inhibition in multiple cancers. *Mol Cancer Res* **15**: 45–58.

- Bellosta P, Costa M, Lin DA, Basilico C (1995) The receptor tyrosine kinase ARK mediates cell aggregation by homophilic binding. *Mol Cell Biol* **15**(2): 614–625.
- Ben-Batalla I, Schultze A, Wroblewski M, Erdmann R, Heuser M, Waizenegger JS, Riecken K, Binder M, Schewe D, Sawall S, Witzke V, Cubas-Cordova M, Janning M, Wellbrock J, Fehse B, Hagel C, Krauter J, Ganser A, Lorens JB, Fiedler W, Carmeliet P, Pantel K, Bokemeyer C, Loges S (2013) Axl, a prognostic and therapeutic target in acute myeloid leukemia mediates paracrine crosstalk of leukemia cells with bone marrow stroma. *Blood* **122**(14): 2443–2452.
- Bosurgi L, Bernink JH, Delgado Cuevas V, Gagliani N, Joannas L, Schmid ET, Booth CJ, Ghosh S, Rothlin CV (2013) Paradoxical role of the proto-oncogene Axl and Mer receptor tyrosine kinases in colon cancer. *Proc Natl Acad Sci USA* **110**(32): 13091–13096.
- Brand TM, Iida M, Stein AP, Corrigan KL, Braverman CM, Coan JP, Pearson HE, Bahrar H, Fowler TL, Bednarz BP, Saha S, Yang D, Gill PS, Lingen MW, Saloura V, Villaflor VM, Salgia R, Kimple RJ, Wheeler DL (2015) AXL is a logical molecular target in head and neck squamous cell carcinoma. *Clin Cancer Res* **21**(11): 2601–2612.
- Brand TM, Iida M, Stein AP, Corrigan KL, Braverman CM, Luthar N, Toulany M, Gill PS, Salgia R, Kimple RJ, Wheeler DL (2014) AXL mediates resistance to cetuximab therapy. *Cancer Res* **74**(18): 5152–5164.
- Braunger J, Schleithoff L, Schulz AS, Kessler H, Lammers R, Ullrich A, Bartram CR, Janssen JW (1997) Intracellular signaling of the Ufo/Axl receptor tyrosine kinase is mediated mainly by a multi-substrate docking-site. *Oncogene* **14**(22): 2619–2631.
- Burchert A, Attar EC, McCloskey P, Fridell YW, Liu ET (1998) Determinants for transformation induced by the Axl receptor tyrosine kinase. *Oncogene* **16**(24): 3177–3187.
- Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, Shen L, Fan Y, Giri U, Tumula PK, Nilsson MB, Gudikote J, Tran H, Cardnell RJ, Bearss DJ, Warner SL, Foulks JM, Kanner SB, Gandhi V, Krett N, Rosen ST, Kim ES, Herbst RS, Blumenschein GR, Lee JJ, Lippman SM, Ang KK, Mills GB, Hong WK, Weinstein JN, Wistuba, Coombes KR, Minna JD, Heymach JV (2013) An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res* **19**(1): 279–290.
- Cancer Genome Atlas N (2015) Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* **517**(7536): 576–582.
- Cancer Genome Atlas Research N (2012) Comprehensive genomic characterization of squamous cell lung cancers. *Nature* **489**(7417): 519–525.
- Cancer Genome Atlas Research N (2013) Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* **368**(22): 2059–2074.
- Cancer Genome Atlas Research N (2014) Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**(7511): 543–550.
- Cichon MA, Szentpetery Z, Caley MP, Papadakis ES, Mackenzie IC, Brennan CH, O'Toole EA (2014) The receptor tyrosine kinase Axl regulates cell-cell adhesion and stemness in cutaneous squamous cell carcinoma. *Oncogene* **33**(32): 4185–4192.
- Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, Bowlby R, Shen H, Hayat S, Fieldhouse R, Lester SC, Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB, Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW, Hoadley KA, King TA, Network TR, Perou CM (2015) Comprehensive molecular portraits of invasive lobular breast cancer. *Cell* **163**(2): 506–519.
- Cui ZL, Han FF, Peng XH, Chen X, Luan CY, Han RC, Xu WG, Guo XJ (2012) YES-associated protein 1 promotes adenocarcinoma growth and metastasis through activation of the receptor tyrosine kinase Axl. *Int J Immunopathol Pharmacol* **25**(4): 989–1001.
- Do KT, MacConaill L, Dubuc AM, Chen I, Chao R, Tassell V, Christensen J, Shapiro GI, Sholl LM (2011) Evaluation of the MET/Axl receptor tyrosine kinase (RTK) inhibitor MGCD265 in a patient with metastatic non-small cell lung cancer (NSCLC) harboring Axl amplification. International Association for the Study of Lung Cancer (IASLC). *Oral Presentation at the 16th World Conference on Lung Cancer*; 6–9 September 2015: Denver, CO, USA.
- Dufies M, Jacquel A, Belhacene N, Robert G, Cluzeau T, Luciano F, Cassuto JP, Raynaud S, Auberger P (2011) Mechanisms of AXL overexpression and function in Imatinib-resistant chronic myeloid leukemia cells. *Oncotarget* **2**(11): 874–885.
- Elkabets M, Pazarentzos E, Juric D, Sheng Q, Pelosof RA, Brook S, Benzaken AO, Rodon J, Morse N, Yan JJ, Liu M, Das R, Chen Y, Tam A, Wang H, Liang J, Gurski JM, Kerr DA, Rosell R, Teixido C, Huang A, Ghossein RA, Rosen N, Bivona TG, Scaltriti M, Baselga J (2015) AXL mediates resistance to PI3Kalpha inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. *Cancer Cell* **27**(4): 533–546.
- Fridell YW, Jin Y, Quilliam LA, Burchert A, McCloskey P, Spizz G, Varnum B, Der C, Liu ET (1996) Differential activation of the Ras/extracellular-signal-regulated protein kinase pathway is responsible for the biological consequences induced by the Axl receptor tyrosine kinase. *Mol Cell Biol* **16**(1): 135–145.
- Fridell YW, Villa Jr J, Attar EC, Liu ET (1998) GAS6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. *J Biol Chem* **273**(12): 7123–7126.
- Fujimori T, Grabiec AM, Kaur M, Bell TJ, Fujino N, Cook PC, Svedberg FR, MacDonald AS, Maciewicz RA, Singh D, Hussell T (2015) The Axl receptor tyrosine kinase is a discriminator of macrophage function in the inflamed lung. *Mucosal Immunol* **8**(5): 1021–1030.
- Gallicchio M, Mitola S, Valdembrì D, Fantozzi R, Varnum B, Avanzi GC, Bussolino F (2005) Inhibition of vascular endothelial growth factor receptor 2-mediated endothelial cell activation by Axl tyrosine kinase receptor. *Blood* **105**(5): 1970–1976.
- Graham DK, DeRyckere D, Davies KD, Earp HS (2014) The TAM family: phosphatidylinositol sensing receptor tyrosine kinases gone awry in cancer. *Nat Rev Cancer* **14**(12): 769–785.
- Han J, Tian R, Yong B, Luo C, Tan P, Shen J, Peng T (2013) Gas6/Axl mediates tumor cell apoptosis, migration and invasion and predicts the clinical outcome of osteosarcoma patients. *Biochem Biophys Res Commun* **435**(3): 493–500.
- Heckmann D, Maier P, Laufs S, Li L, Sleeman JP, Trunk MJ, Leupold JH, Wenz F, Zeller WJ, Fruehauf S, Allgayer H (2014) The disparate twins: a comparative study of CXCR4 and CXCR7 in SDF-1alpha-induced gene expression, invasion and chemosensitivity of colon cancer. *Clin Cancer Res* **20**(3): 604–616.
- Hong CC, Lay JD, Huang JS, Cheng AL, Tang JL, Lin MT, Lai GM, Chuang SE (2008) Receptor tyrosine kinase AXL is induced by chemotherapy drugs and overexpression of AXL confers drug resistance in acute myeloid leukemia. *Cancer Lett* **268**(2): 314–324.
- Hsieh MS, Yang PW, Wong LF, Lee JM (2016) The AXL receptor tyrosine kinase is associated with adverse prognosis and distant metastasis in esophageal squamous cell carcinoma. *Oncotarget* **7**(24): 36956–36970.
- Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskova S, Berent-Maoz B, Pang J, Chmielowski B, Cherry G, Seja E, Lomeli S, Kong X, Kelley MC, Sosman JA, Johnson DB, Ribas A, Lo RS (2016) Genomic and transcriptomic features of response to Anti-PD-1 therapy in metastatic melanoma. *Cell* **165**(1): 35–44.
- Ishikawa M, Sonobe M, Nakayama E, Kobayashi M, Kikuchi R, Kitamura J, Imamura N, Date H (2013) Higher expression of receptor tyrosine kinase Axl, and differential expression of its ligand, Gas6, predict poor survival in lung adenocarcinoma patients. *Ann Surg Oncol* **20**(Suppl 3): S467–S476.
- Janssen JW, Schulz AS, Steenvoorden AC, Schmidberger M, Strehl S, Ambros PF, Bartram CR (1991) A novel putative tyrosine kinase receptor with oncogenic potential. *Oncogene* **6**(11): 2113–2120.
- Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, Zaritskey A, Countouriotis A, Besson N, Leip E, Kelly V, Brummendorf TH (2012) Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. *Blood* **119**(15): 3403–3412.
- Kim KC, Baek SH, Lee C (2015) Curcumin-induced downregulation of Axl receptor tyrosine kinase inhibits cell proliferation and circumvents chemoresistance in non-small lung cancer cells. *Int J Oncol* **47**(6): 2296–2303.
- Konieczkowski DJ, Johannessen CM, Abudayyeh O, Kim JW, Cooper ZA, Piris A, Frederick DT, Barzily-Rokni M, Straussman R, Haq R, Fisher DE, Mesirov JP, Hahn WC, Flaherty KT, Wargo JA, Tamayo P, Garraway LA (2014) A melanoma cell state distinction influences sensitivity to MAPK pathway inhibitors. *Cancer Discov* **4**(7): 816–827.
- Koorstra JB, Karikari CA, Feldmann G, Bisht S, Rojas PL, Offerhaus GJ, Alvarez H, Maitra A (2009) The Axl receptor tyrosine kinase confers an adverse prognostic influence in pancreatic cancer and represents a new therapeutic target. *Cancer Biol Ther* **8**(7): 618–626.

- Lantin le Boulch N, Truong-Ngoc NA, Gauchy C, Besson MJ (1991) In vivo release of newly synthesized [3H]GABA in the substantia nigra of the rat: relative contribution of GABA striato-pallido-nigral afferents and nigral GABA neurons. *Brain Res* **559**(2): 200–210.
- Lay JD, Hong CC, Huang JS, Yang YY, Pao CY, Liu CH, Lai YP, Lai GM, Cheng AL, Su IJ, Chuang SE (2007) Sulfasalazine suppresses drug resistance and invasiveness of lung adenocarcinoma cells expressing AXL. *Cancer Res* **67**(8): 3878–3887.
- Lee CH, Yen CY, Liu SY, Chen CK, Chiang CF, Shiah SG, Chen PH, Shieh YS (2012) Axl is a prognostic marker in oral squamous cell carcinoma. *Ann Surg Oncol* **19**(Suppl 3): S500–S508.
- Lee HJ, Jeng YM, Chen YL, Chung L, Yuan RH (2014) Gas6/Axl pathway promotes tumor invasion through the transcriptional activation of Slug in hepatocellular carcinoma. *Carcinogenesis* **35**(4): 769–775.
- Li N, Liu Z, Zhang Y, Chen Q, Liu P, Cheng CY, Lee WM, Chen Y, Han D (2015) Mice lacking Axl and Mer tyrosine kinase receptors are susceptible to experimental autoimmune orchitis induction. *Immunol Cell Biol* **93**(3): 311–320.
- Liu E, Hjelle B, Bishop JM (1988) Transforming genes in chronic myelogenous leukemia. *Proc Natl Acad Sci USA* **85**(6): 1952–1956.
- Liu L, Greger J, Shi H, Liu Y, Greshock J, Annan R, Halsey W, Sathe GM, Martin AM, Gilmer TM (2009) Novel mechanism of lapatinib resistance in HER2-positive breast tumor cells: activation of AXL. *Cancer Res* **69**(17): 6871–6878.
- Lozneanu L, Pinciroli P, Ciobanu DA, Carcangiu ML, Canevari S, Tomassetti A, Caruntu ID (2016) Computational and immunohistochemical analyses highlight AXL as a potential prognostic marker for ovarian cancer patients. *Anticancer Res* **36**(8): 4155–4163.
- Mackiewicz M, Huppi K, Pitt JJ, Dorsey TH, Ambs S, Caplen NJ (2011) Identification of the receptor tyrosine kinase AXL in breast cancer as a target for the human miR-34a microRNA. *Breast Cancer Res Treat* **130**(2): 663–679.
- Macleod K, Mullen P, Sewell J, Rabiasz G, Lawrie S, Miller E, Smyth JF, Langdon SP (2005) Altered ErbB receptor signaling and gene expression in cisplatin-resistant ovarian cancer. *Cancer Res* **65**(15): 6789–6800.
- Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, Wisner I, Iorio M, Shakalya K, Garewal H, Nagle R, Bearss D (2007) A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. *Oncogene* **26**(27): 3909–3919.
- Mahadevan D, Theiss N, Morales C, Stejskal AE, Cooke LS, Zhu M, Kurtzman D, Swart R, Ong E, Qi W (2015) Novel receptor tyrosine kinase targeted combination therapies for imatinib-resistant gastrointestinal stromal tumors (GIST). *Oncotarget* **6**(4): 1954–1966.
- Mak MP, Tong P, Diao L, Cardnell RJ, Gibbons DL, William WN, Skoulidis F, Parra ER, Rodriguez-Canales J, Wistuba, Heymach JV, Weinstein JN, Coombes KR, Wang J, Byers LA (2016) A patient-derived, pan-cancer EMT signature identifies global molecular alterations and immune target enrichment following epithelial-to-mesenchymal transition. *Clin Cancer Res* **22**(3): 609–620.
- Meyer AS, Miller MA, Gertler FB, Lauffenburger DA (2013) The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. *Sci Signal* **6**(287): ra66.
- Meyer AS, Zweemer AJ, Lauffenburger DA (2015) The AXL receptor is a sensor of ligand spatial heterogeneity. *Cell Syst* **1**(1): 25–36.
- Mudduluru G, Allgayer H (2008) The human receptor tyrosine kinase Axl gene—promoter characterization and regulation of constitutive expression by Sp1, Sp3 and CpG methylation. *Biosci Rep* **28**(3): 161–176.
- Mudduluru G, Ceppi P, Kumarswamy R, Scagliotti GV, Papotti M, Allgayer H (2011) Regulation of Axl receptor tyrosine kinase expression by miR-34a and miR-199a/b in solid cancer. *Oncogene* **30**(25): 2888–2899.
- Mudduluru G, Leupold JH, Stroebel P, Allgayer H (2010a) PMA up-regulates the transcription of Axl by AP-1 transcription factor binding to TRE sequences via the MAPK cascade in leukaemia cells. *Biol Cell* **103**(1): 21–33.
- Mudduluru G, Vajkoczy P, Allgayer H (2010b) Myeloid zinc finger 1 induces migration, invasion, and in vivo metastasis through Axl gene expression in solid cancer. *Mol Cancer Res* **8**(2): 159–169.
- Muller J, Krijgsman O, Tsoi J, Robert L, Hugo W, Song C, Kong X, Possik PA, Cornelissen-Steyger PD, Geukes Foppen MH, Kemper K, Goding CR, McDermott U, Blank C, Haanen J, Graeber TG, Ribas A, Lo RS, Peeper DS (2014) Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. *Nat Commun* **5**: 5712.
- Neubauer A, Fiebler A, Graham DK, O'Bryan JP, Schmidt CA, Barckow P, Serke S, Siegert W, Snodgrass HR, Huhn D, Liu ET (1994) Expression of axl, a transforming receptor tyrosine kinase, in normal and malignant hematopoiesis. *Blood* **84**(6): 1931–1941.
- Nguyen KQ, Tsou WI, Kottenko S, Birge RB (2013) TAM receptors in apoptotic cell clearance, autoimmunity, and cancer. *Autoimmunity* **46**(5): 294–297.
- Nowakowski TJ, Pollen AA, Di Lullo E, Sandoval-Espinosa C, Bershteyn M, Kriegstein AR (2016) Expression analysis highlights AXL as a candidate zika virus entry receptor in neural stem cells. *Cell Stem Cell* **18**(5): 591–596.
- O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, Espinosa 3rd R, Le Beau MM, Earp HS, Liu ET (1991) AXL, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol* **11**(10): 5016–5031.
- O'Donnell K, Harkes IC, Dougherty L, Wicks IP (1999) Expression of receptor tyrosine kinase Axl and its ligand Gas6 in rheumatoid arthritis: evidence for a novel endothelial cell survival pathway. *Am J Pathol* **154**(4): 1171–1180.
- Onken J, Torka R, Korsing S, Radke J, Kremeskaia I, Nieminen M, Bai X, Ullrich A, Heppner F, Vajkoczy P (2016) Inhibiting receptor tyrosine kinase AXL with small molecule inhibitor BMS-777607 reduces glioblastoma growth, migration, and invasion *in vitro* and *in vivo*. *Oncotarget* **7**(9): 9876–9889.
- Ott M, Litzenburger UM, Sahm F, Rauschenbach KJ, Tudoran R, Hartmann C, Marquez VE, von Deimling A, Wick W, Platten M (2012) Promotion of glioblastoma cell motility by enhancer of zeste homolog 2 (EZH2) is mediated by AXL receptor kinase. *PLOS One* **7**(10): e47663.
- Ou WB, Corson JM, Flynn DL, Lu WP, Wise SC, Bueno R, Sugarbaker DJ, Fletcher JA (2011) AXL regulates mesothelioma proliferation and invasiveness. *Oncogene* **30**(14): 1643–1652.
- Pacez JD, Vasquez GJ, Correa RG, Vasconcellos JF, Duncan K, Gu X, Bhasin M, Libermann TA, Zerbini LF (2013) The receptor tyrosine kinase Axl is an essential regulator of prostate cancer proliferation and tumor growth and represents a new therapeutic target. *Oncogene* **32**(6): 689–698.
- Paolino M, Choidas A, Wallner S, Pranjic B, Uribealago I, Loeser S, Jamieson AM, Langdon WY, Ikeda F, Fededa JP, Cronin SJ, Nitsch R, Schultz-Fademrecht C, Eickhoff J, Menninger S, Unger A, Torka R, Gruber T, Hinterleitner R, Baier G, Wolf D, Ullrich A, Klebl BM, Penninger JM (2014) The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* **507**(7493): 508–512.
- Perera-Lecoin M, Meertens L, Carne X, Amara A (2014) Flavivirus entry receptors: an update. *Viruses* **6**(1): 69–88.
- Rankin EB, Fuh KC, Castellini L, Viswanathan K, Finger EC, Diep AN, LaGory EL, Kariolis MS, Chan A, Lindgren D, Axelson H, Miao YR, Krieg AJ, Giaccia AJ (2014) Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. *Proc Natl Acad Sci USA* **111**(37): 13373–13378.
- Ravichandran KS (2010) Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. *J Exp Med* **207**(9): 1807–1817.
- Read SA, Tay ES, Shahidi M, O'Connor K, Booth DR, George J, Douglas MW (2015) Hepatitis C Virus Driven AXL Expression suppresses the Hepatic Type I Interferon Response. *PLOS ONE* **10**(8): e0136227.
- Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G (2007) TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* **131**(6): 1124–1136.
- Ruan GX, Kazlauskas A (2012) Axl is essential for VEGF-A-dependent activation of PI3K/Akt. *EMBO J* **31**(7): 1692–1703.
- Ruan GX, Kazlauskas A (2013) Lactate engages receptor tyrosine kinases Axl, Tie2, and vascular endothelial growth factor receptor 2 to activate phosphoinositide 3-kinase/Akt and promote angiogenesis. *J Biol Chem* **288**(29): 21161–21172.
- Sainaghi PP, Castello L, Bergamasco L, Galletti M, Bellosa P, Avanzi GC (2005) Gas6 induces proliferation in prostate carcinoma cell lines expressing the Axl receptor. *J Cell Physiol* **204**(1): 36–44.
- Sasaki T, Knyazev PG, Clout NJ, Cheburkin Y, Gohring W, Ullrich A, Timpl R, Hohenester E (2006) Structural basis for Gas6-Axl signalling. *EMBO J* **25**(1): 80–87.
- Seo JS, Ju YS, Lee WC, Shin JY, Lee JK, Bleazard T, Lee J, Jung YJ, Kim JO, Shin JY, Yu SB, Kim J, Lee ER, Kang CH, Park IK, Rhee H, Lee SH, Kim JI, Kang JH, Kim YT (2012) The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome Res* **22**(11): 2109–2119.
- Sharif MN, Sosic D, Rothlin CV, Kelly E, Lemke G, Olson EN, Ivashkiv LB (2006) Twist mediates suppression of inflammation by type I IFNs and Axl. *J Exp Med* **203**(8): 1891–1901.

- Shieh YS, Lai CY, Kao YR, Shiah SG, Chu YW, Lee HS, Wu CW (2005) Expression of axl in lung adenocarcinoma and correlation with tumor progression. *Neoplasia* 7(12): 1058–1064.
- Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Mattsson K, Fisher J, Gies DR, Jones PF, Masiakowski P, Ryan TE, Tobkes NJ, Chen DH, DiStefano PS, Long GL, Basilico C, Goldfarb MP, Lemke G, Glass DJ, Yancopoulos GD (1995) The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. *Cell* 80(4): 661–670.
- Tai KY, Shieh YS, Lee CS, Shiah SG, Wu CW (2008) Axl promotes cell invasion by inducing MMP-9 activity through activation of NF-kappaB and Brg-1. *Oncogene* 27(29): 4044–4055.
- Tanaka K, Tokunaga E, Inoue Y, Yamashita N, Saeki H, Okano S, Kitao H, Oki E, Oda Y, Maehara Y (2016) Impact of expression of vimentin and Axl in breast cancer. *Clin Breast Cancer* 16(6): 520–526.e2.
- Tirosch I, Izar B, Prakadan SM, Wadsworth 2nd MH, Treacy D, Trombetta JJ, Rotem A, Rodman C, Lian C, Murphy G, Fallahi-Sichani M, Dutton-Regester K, Lin JR, Cohen O, Shah P, Lu D, Genshaft AS, Hughes TK, Ziegler CG, Kazer SW, Gaillard A, Kolb KE, Villani AC, Johannessen CM, Andreev AY, Van Allen EM, Bertagnolli M, Sorger PK, Sullivan RJ, Flaherty KT, Frederick DT, Jane-Valbuena J, Yoon CH, Rozenblatt-Rosen O, Shalek AK, Regev A, Garraway LA (2016) Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352(6282): 189–196.
- Toledo RA, Qin Y, Cheng ZM, Gao Q, Iwata S, Silva GM, Prasad ML, Ocal IT, Rao S, Aronin N, Barontini M, Bruder J, Reddick RL, Chen Y, Aguiar RC, Dahia PL (2016) Recurrent mutations of chromatin-remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. *Clin Cancer Res* 22(9): 2301–2310.
- Varnum BC, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW, Hunt RW, Trail G, Clogston C, Toso RJ, Yanagihara D, Bennett L, Sylber M, Merewether LA, Tseng A, Escobar E, Liu ET, Yamane HK (1995) Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. *Nature* 373(6515): 623–626.
- Vouri M, Croucher DR, Kennedy SP, An Q, Pilkington GJ, Hafizi S (2016) Axl-EGFR receptor tyrosine kinase hetero-interaction provides EGFR with access to pro-invasive signalling in cancer cells. *Oncogenesis* 5(10): e266.
- Wang C, Jin H, Wang N, Fan S, Wang Y, Zhang Y, Wei L, Tao X, Gu D, Zhao F, Fang J, Yao M, Qin W (2016) Gas6/Axl axis contributes to chemoresistance and metastasis in breast cancer through Akt/GSK-3beta/beta-catenin signaling. *Theranostics* 6(8): 1205–1219.
- Wang X, Saso H, Iwamoto T, Xia W, Gong Y, Pusztai L, Woodward WA, Reuben JM, Warner SL, Bearss DJ, Hortobagyi GN, Hung MC, Ueno NT (2013) TIG1 promotes the development and progression of inflammatory breast cancer through activation of Axl kinase. *Cancer Res* 73(21): 6516–6525.
- Weinger JG, Brosnan CF, Loudig O, Goldberg MF, Macian F, Arnett HA, Prieto AL, Tsiperson V, Shafit-Zagardo B (2011) Loss of the receptor tyrosine kinase Axl leads to enhanced inflammation in the CNS and delayed removal of myelin debris during experimental autoimmune encephalomyelitis. *J Neuroinflammation* 8: 49.
- Ye X, Li Y, Stawicki S, Couto S, Eastham-Anderson J, Kallop D, Weimer R, Wu Y, Pei L (2010) An anti-Axl monoclonal antibody attenuates xenograft tumor growth and enhances the effect of multiple anticancer therapies. *Oncogene* 29(38): 5254–5264.
- Yuen HF, McCrudden CM, Huang YH, Tham JM, Zhang X, Zeng Q, Zhang SD, Hong W (2013) TAZ expression as a prognostic indicator in colorectal cancer. *PLOS One* 8(1): e54211.
- Zagorska A, Traves PG, Lew ED, Dransfield I, Lemke G (2014) Diversification of TAM receptor tyrosine kinase function. *Nat Immunol* 15(10): 920–928.
- Zhang Y, Tang YJ, Man Y, Pan F, Li ZH, Jia LS (2013) Knockdown of AXL receptor tyrosine kinase in osteosarcoma cells leads to decreased proliferation and increased apoptosis. *Int J Immunopathol Pharmacol* 26(1): 179–188.
- Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T, Abdel-Rahman M, Wang X, Levine AD, Rho JK, Choi YJ, Choi CM, Kim SW, Jang SJ, Park YS, Kim WS, Lee DH, Lee JS, Miller VA, Arcila M, Ladanyi M, Moonsamy P, Sawyers C, Boggon TJ, Ma PC, Costa C, Taron M, Rosell R, Halmos B, Bivona TG (2012) Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* 44(8): 852–860.
- Zhou L, Liu XD, Sun M, Zhang X, German P, Bai S, Ding Z, Tannir N, Wood CG, Matin SF, Karam JA, Tamboli P, Sircar K, Rao P, Rankin EB, Laird DA, Hoang AG, Walker CL, Giaccia AJ, Jonasch E (2016) Targeting MET and AXL overcomes resistance to sunitinib therapy in renal cell carcinoma. *Oncogene* 35(21): 2687–2697.



This work is licensed under the Creative Commons Attribution-Non-Commercial-Share Alike 4.0 International License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/4.0/>

© The Author(s) named above 2017