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Giving AXL the axe: targeting AXL in human malignancy

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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

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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-κB, and JAK/STAT (Fridell *et al*, 1996; Tai *et al*, 2008; Ruan and Kazlauskas, 2012; Paccez *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

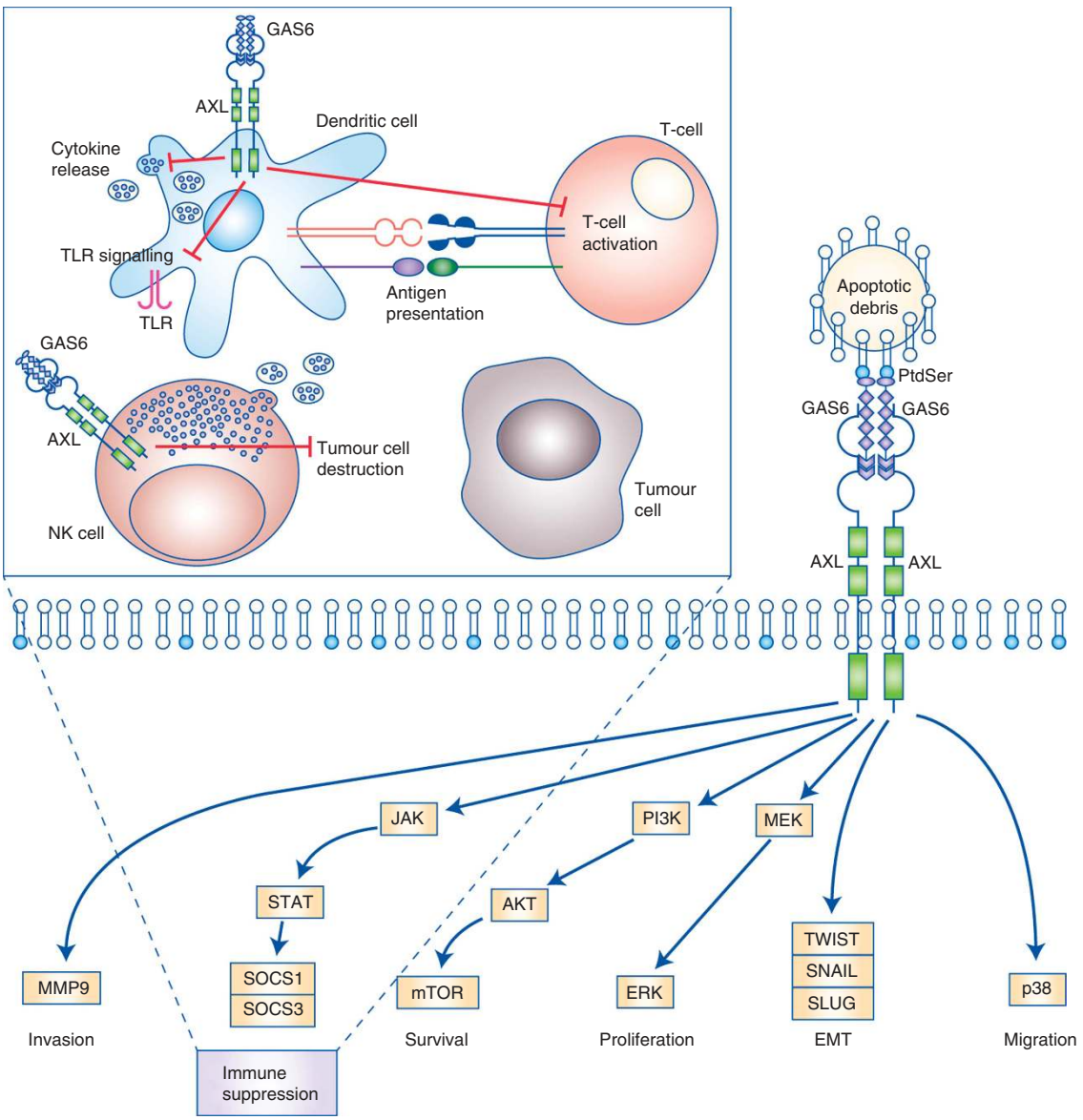

Figure 1. Spectrum of cellular processes regulated by AXL activity. AXL, following activation by its ligand GAS6 along with an interaction between GAS6 and phosphatidylserine (PtdSer), dimerises and cross-phosphorylates (yellow circle) its partner receptor. This activation regulates an array of cellular 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 HIF1α 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).

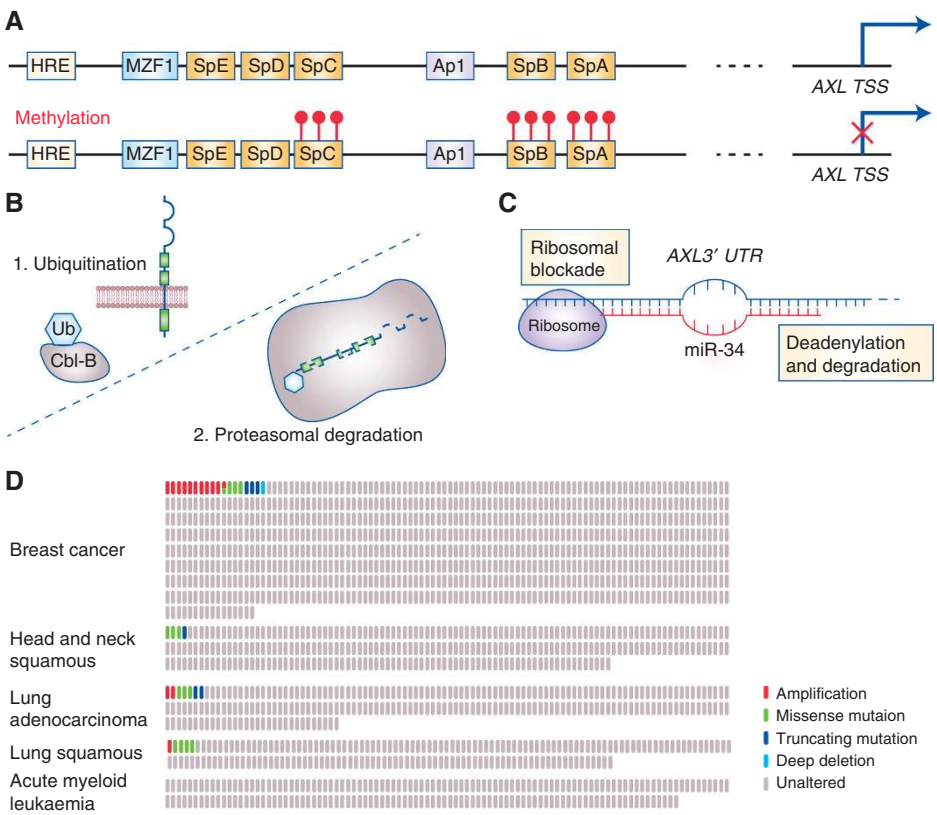

Figure 2. Transcriptional and post-transcriptional regulation of *AXL*/AXL. (**A**) The promoter region upstream of the *AXL* transcriptional start site (TSS) highlights binding sites for transcription factors, including hypoxia responsive element (HRE) for HIF1α, MZF1, and AP1. The promoter region also 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-κB. 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-κB-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 TGFβ-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, HIF1α, 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 PLCγ (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 ± 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 (Lozneanu *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 (Khoury *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, PDGFRβ, 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 (GL2I.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.

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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.

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