

## **TAM receptor tyrosine kinases in cancer drug resistance**

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## **Abstract**

Receptor tyrosine kinases (RTKs) are major regulators of key biological processes including cell growth, survival and differentiation, and were established early on as proto-oncogenes, with aberrant expression linked to tumor progression in many cancers. Therefore, RTKs have emerged as major targets for selective therapy with small molecule inhibitors. However, despite improvements in survival rates, it is now apparent that targeting of RTKs with selective inhibitors is only transiently effective as the majority of patients eventually become resistant to therapy. As chemoresistance is the leading cause of cancer spread, progression and mortality, there is an increasing need for understanding the mechanisms by which cancer cells can evade therapy-induced cell death. The TAM (Tyro3, Axl, Mer) subfamily of RTKs in particular feature in a variety of cancer types that have developed resistance to a broad range of therapeutic agents, including both targeted as well as conventional chemotherapeutics. This article reviews the roles of TAMs as tumor drivers and as mediators of chemoresistance, and the potential effectiveness of targeting them as part of therapeutic strategies to delay or combat resistance.

## **Introduction**

The TAM (Tyro3, Axl, MerTK) family of receptor tyrosine kinases (RTKs) is defined by each member possessing an extracellular combination of two immunoglobulin-like domains and two fibronectin type III repeats, a transmembrane portion, and an intracellular region with intrinsic tyrosine kinase activity. The human TAM genes share similar genomic structures, and their resulting proteins share significant structural similarities, with greatest homology in the tyrosine kinase domain. The apparent molecular weights range from 100-140 kDa for Axl and Tyro3 and 165–205 kDa for MerTK due to post-translational

modifications, including glycosylation, phosphorylation and ubiquitination. The established natural ligands for the TAMs are two homologous vitamin K-dependent proteins, Gas6 (all three TAMs) and Protein S (Tyro3 and MerTK) (1).

## **TAM functions**

All three TAMs have transforming potential; however, Axl overexpression has most frequently been detected in multiple cancers and its role in supporting tumorigenesis is well recognized. Axl supports tumour growth and dissemination through positive effects on cell survival, proliferation, migration, and invasion. Alongside these, Axl signaling is also involved in other processes ranging from the differentiation of cells in the erythroid lineage, protecting blood vessels from injury, clearance of apoptotic cells, angiogenesis, hematopoiesis, platelet aggregation and regulation of pro-inflammatory cytokine production (2, 3). MerTK is most distinctly recognized for its role in negative regulation of the immune system through its ability to mediate phagocytosis of apoptotic cells (4). Tyro3 has a more restricted tissue expression profile, and has been implicated in various roles including myelination in the brain (5).

## **The TAMs as mediators of chemoresistance**

Although the TAMs are not strong oncogenes, it is increasingly clear that their overexpression contributes to acquiring resistance to both conventional as well as targeted chemotherapeutics in both solid and blood cancers. Early observations included increased Axl overexpression with resistance to the small molecule inhibitor imatinib (targets BCR-Abl, c-Kit and PDGFR) in chronic myelogenous leukemia (CML) (6) and to the chemotherapy drugs doxorubicin, VP16 and cisplatin in acute myeloid leukemia (7).

Increased resistance to imatinib due to Axl and MerTK overexpression has also been reported in gastrointestinal stromal tumors (8) and non-small lung carcinoma (9).

MerTK overexpression has been detected in high grade glioblastomas and its knockdown to result in increased sensitivity to etoposide whilst, conversely, control cells showed elevated MerTK activation upon DNA damage with increase resistance to etoposide (10). Inhibition of Axl and MerTK also lead to increased chemosensitivity to temozolomide, carboplatin and vincristine in astrocytoma (11), and to cisplatin and vincristine in neuroblastoma (12). Axl was also shown to promote resistance to ALK inhibitors in neuroblastoma through induction of epithelial-mesenchymal transition (EMT) (13). MerTK inhibition by a monoclonal antibody was able to increase sensitivity to carboplatin in non-small cell lung cancer (NSCLC) due to receptor internalization and subsequent degradation which led to STAT6, Akt and ERK1/2 signaling inhibition (14). MerTK was also shown in NSCLC to be essential for increased resistance to erlotinib through the regulation of MAPK and FAK signaling pathways (15).

Knockdown of Axl by short hairpin (sh)RNA has revealed Axl to promote the survival of cutaneous squamous carcinoma cells through Akt activation and inhibition of the pro-apoptotic Bcl-2 family proteins (16). Recently in melanoma, Tyro3 was identified to regulate expression of Microphthalmia-associated transcription factor (MITF), a master regulator of melanocyte development, and to promote tumorigenesis in spite of B-Raf-induced senescence (17). Tyro3 was also shown to promote cell proliferation and chemoresistance in breast cancer (18). In addition, downregulation of both Axl and Tyro3 was able to reverse taxol resistance in ovarian cancer (19).

Increased Axl expression has also been associated with acquired resistance to selective EGFR small molecule inhibition. In lung cancers, this includes resistance to erlotinib through the induction of an EMT-like state (20), as well as to gefitinib (21). In

triple-negative breast cancer cells, expression of Axl was identified as a predictor for lack of response to lapatinib and erlotinib (22). Targeting EGFR antibodies such as cetuximab has also lead to resistance in NSCLC and head and neck carcinomas through Axl overexpression, via MAPK signaling (23). Such an association between EGFR and Axl molecular pathways also manifests itself through hetero-interaction between EGFR and Axl molecules, which can diversify downstream signaling pathways beyond those triggered by EGFR alone (24).

The prominent expression and functions of TAM receptors across the immune system also suggests a potential role in mediating resistance to immune checkpoint inhibitors in cancer therapy. TAM receptors are notably expressed in many myeloid immune cells including macrophages and dendritic cells, and they may cooperate to create an immunosuppressive tumor microenvironment permissive to tumorigenesis (25). MerTK is implicated in the suppression of the M1 macrophage pro-inflammatory cytokine response, and Axl is required for the termination of Toll-like receptor dependent inflammatory response in dendritic cells (25). Also, the TAMs have been shown to negatively regulate NK cell functional maturation and normal expression of inhibitory and activating NK cell receptors (26). Furthermore, TAM receptors promote cancer metastases *in vivo* through suppression of NK cell activity, whilst treatment of wild-type NK cells with a TAM-selective small molecule inhibitor enhanced anti-metastatic NK cell activity (27). Collectively, these data implicate TAMs as key players in regulation of the innate immune system in the tumor microenvironment and indicate that their targeted inhibition will reverse the immunosuppressive microenvironment. This would in particular be expected to have an impact with use of immune checkpoint blockers in cancer therapy, e.g. inhibitors of CTLA-4, PD-1 or PD-L1. The TAMs, in particular MerTK, have been shown to upregulate the immune checkpoint molecule PD-L1 as well as promoting phosphatidylserine-dependent efferocytosis and Akt-mediated chemoresistance in tumor cells (28). Therefore, targeting TAM receptors

in combination with immune checkpoint blockers could both enhance the latter's efficacy as well as combat eventual resistance. In support of this, recent data from mouse models of carcinoma showed the Axl inhibitor BGB324 in combination with immune checkpoint inhibitors to enhance tumour clearance, survival and tumour infiltration of cytotoxic T cells (29).

Other influences that can contribute further to TAM-mediated tumor chemoresistance could include increased expression TAM ligands, although this is not clear. Gas6 expression has been identified as a marker for poor overall survival in ovarian cancer (30). Also, galectin-3, a sugar-binding protein that has been implicated in cancer cell behaviors (31), was indicated to be a novel ligand for MerTK (4). Axl expression has also been correlated with markers of DNA repair in different solid cancers; Axl inhibition induced reversal of EMT and decreased expression of DNA repair genes, rendering cancer cells sensitive to PARP inhibition in a synergistic manner (32).

### **TAM inhibition to treat tumour drug resistance**

In addition to their roles in primary tumorigenesis, the prevalence of TAM receptors in patients displaying acquired drug-resistant tumors renders the TAMs a viable target for therapies against such evolved tumours. Importantly, TAM signaling inhibition sensitizes cells to chemotherapy, indicating that its effects are multifaceted, and the aim of “shutting down” multiple key biological processes in cancer cells can be achieved through a single target. A plethora of new, more specific and higher affinity targeting agents are currently under development (Table 1) that may prove to be effective for preventing, delaying or combating tumour drug resistance.

**Table 1:** TAM-selective inhibitors currently in development. All are small molecule inhibitors except YW327.6S2, which is a monoclonal antibody and GL21.T, which is an RNA aptamer.

<b>Name</b>	<b>Target(s)</b>	<b>Development stage</b>
<b>DP3975</b>	Axl	Pre-clinical
<b>LDC1267</b>	Axl, Tyro3, Mer	Pre-clinical
<b>NA80x1</b>	Axl	Pre-clinical
<b>YW327.6S2</b>	Axl	Pre-clinical
<b>GL21.T</b>	Axl	Pre-clinical
<b>NPS-1034</b>	Axl, c-Met	Pre-clinical
<b>UNC1062</b>	Mer	Pre-clinical
<b>UNC569</b>	Mer	Pre-clinical
<b>BGB324</b>	Axl	Phase I clinical trial for NSCLC (NCT02922777), AML (NCT02488408) and metastatic melanoma (NCT02872259)
<b>BMS777607</b>	c-Met, Ron, Flt-3, Tyro3 Mer, Axl	Phase I clinical trial for advanced and/or metastatic solid tumors (NCT01721148)
<b>Glesatinib (MGCD265)</b>	Axl, c-Met	Phase II clinical trial for NSCLC patients with activating mutations in <i>MET</i> (NCT02544633).
<b>Sunitinib (SU11248)</b>	Flt-3, Axl, VEGFR-2, Kit	Approved for pancreatic neuroendocrine and gastrointestinal stromal tumor and kidney cancer.
<b>Cabozantinib</b>	VEGFR-2, c-Met, Ret, Kit, Flt-3, Axl	Approved for medullary thyroid cancer.

A recent example is from a study that showed Axl to be upregulated in CML patients that had developed resistance to BCR-Abl small molecule inhibition, including through a T315I mutation in BCR-Abl. The selective Axl small molecule inhibitor BGB324 inhibited the resistant CML cells independent of BCR-Abl mutational status (33). This example

indicates an efficacious employment of Axl inhibition at the earliest signs of resistance development to first line or targeted therapies.

## **Potential concerns about TAM inhibition**

The broad cellular and tissue expression profile of TAMs and their roles in the maintenance of the bodily organs' normal functions should be a consideration in strategies aimed at inhibiting TAM receptors in cancer therapy. In particular, the role of TAMs in systemic immunity may represent a double-edged sword in terms of clinical outcome in strategies principally aimed at bolstering anti-tumor immunity. Both Axl and MerTK are expressed on multiple immune cells, such as dendritic cells and macrophages and have an essential immune-suppressive role via the abrogation of Toll-like receptor and cytokine receptor signaling. Sustained immune activation and chronic inflammation occurs in TAM triple knockout mice (34), and some cytokine receptor signaling systems appear co-dependent on TAM receptors (35). Additionally, one or more of the TAM receptors is a key gateway for phagocytic clearance of apoptotic cells or fragments, as observed in retinal degeneration due to MerTK impairment and hence defective uptake of photoreceptor outer segments by the retinal pigment epithelium (36). Experimental Mer blockade has also been shown to result in a greater proportion of dendritic cells with enhanced T cell activation capacity versus tolerogenic dendritic cells (37). Thus, prolonged TAM inhibition, through blocking clearance of dying cancer cells and their subsequent build-up, could result in adverse immune or inflammatory reactions beyond those specifically directed at the tumour cells. Also, Axl and MerTK double knockout mice displayed enhanced colitis resulting from insufficient neutrophil clearance (25), and an increased production of pro-inflammatory cytokines favoring a tumor-promoting environment with enhanced colonic polyps formation (38).



Results from current clinical trials with multikinase inhibitors including the TAMs (Table 1) already point to a weakened immune system and accumulation of lymphoid cells. This mirrors that which is observed in TAM knockout mice, in which activation of dendritic cells and macrophages also occurs, as well as clinical signs featured in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (39). Therefore, therapeutic TAM targeting should consider carefully the possibility of inciting adverse pro-inflammatory effects that may conversely favor tumor promotion as opposed to a desired anti-tumor immune response. A particularly effective approach, which warrants clinical trial evaluation, would be TAM inhibition in combination with immune checkpoint blockade to target resistance as well as stimulate anti-tumour immunity specifically.

The TAM RTKs represent a distinct grouping of novel anti-cancer targets through their promotion of tumor cell survival, proliferation, invasion and chemoresistance, as well as suppression of the immune status of the tumour microenvironment. Therefore, therapeutic TAM inhibition may sensitize tumor cells to killing by chemotherapy, radiation or other targeted agents and, in doing so, may create an even more robust innate immune response, which may enhance immunotherapeutic efficacy in combination with immune checkpoint inhibitors. Although development of autoimmunity is a consideration for any sustained TAM inhibition protocol, nevertheless the employment of novel selective TAM inhibitors has major potential moving forward in combating anti-cancer drug resistance for many solid and hematological malignancies.

## References

1. Hafizi S, Dahlbäck B. Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily. *FEBS J.* 2006;273:5231-44.
2. Hafizi S, Dahlbäck B. Signalling and functional diversity within the Axl subfamily of receptor tyrosine kinases. *Cytokine & Growth Factor Reviews.* 2006;17:295-304.

3. Graham DK, DeRyckere D, Davies KD, Earp HS. The TAM family: phosphatidylserine-sensing receptor tyrosine kinases gone awry in cancer. *Nat Rev Cancer*. 2014;14:769-85.
4. Caberoy NB, Alvarado G, Bigcas JL, Li W. Galectin-3 is a new MerTK-specific eat-me signal. *J Cell Physiol*. 2012;227:401-7.
5. Goudarzi S, Rivera A, Butt AM, Hafizi S. Gas6 Promotes Oligodendrogenesis and Myelination in the Adult Central Nervous System and After Lysolecithin-Induced Demyelination. *ASN neuro*. 2016;8.
6. Dufies M, Jacquelin A, Belhacene N, Robert G, Cluzeau T, Luciano F, et al. Mechanisms of AXL overexpression and function in Imatinib-resistant chronic myeloid leukemia cells 2011.
7. Hong C-C, Lay J-D, Huang J-S, Cheng A-L, Tang J-L, Lin M-T, et al. Receptor tyrosine kinase AXL is induced by chemotherapy drugs and overexpression of AXL confers drug resistance in acute myeloid leukemia. *Cancer Letters*. 2008;268:314-24.
8. Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, et al. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. *Oncogene*. 2007;26:3909-19.
9. Linger RMA, Cohen RA, Cummings CT, Sather S, Migdall-Wilson J, Middleton DHG, et al. Mer or Axl receptor tyrosine kinase inhibition promotes apoptosis, blocks growth and enhances chemosensitivity of human non-small cell lung cancer. *Oncogene*. 2012.
10. Wang Y, Moncayo G, Morin P, Jr., Xue G, Grzmil M, Lino MM, et al. Mer receptor tyrosine kinase promotes invasion and survival in glioblastoma multiforme. *Oncogene*. 2012.
11. Keating AK, Kim GK, Jones AE, Donson AM, Ware K, Mulcahy JM, et al. Inhibition of Mer and Axl Receptor Tyrosine Kinases in Astrocytoma Cells Leads to Increased Apoptosis and Improved Chemosensitivity. *Molecular Cancer Therapeutics*. 2010;9:1298-307.
12. Li Y, Wang X, Bi S, Zhao K, Yu C. Inhibition of Mer and Axl receptor tyrosine kinases leads to increased apoptosis and improved chemosensitivity in human neuroblastoma. *Biochemical and Biophysical Research Communications*. 2015;457:461-6.
13. Debruyne DN, Bhatnagar N, Sharma B, Luther W, Moore NF, Cheung N-K, et al. ALK inhibitor resistance in ALK(F1174L)-driven neuroblastoma is associated with AXL activation and induction of EMT. *Oncogene*. 2015;10.1038/onc.2015.434.
14. Cummings CT, Linger RMA, Cohen RA, Sather S, Kirkpatrick GD, Davies KD, et al. Mer590, a novel monoclonal antibody targeting MER receptor tyrosine kinase, decreases colony formation and increases chemosensitivity in non-small cell lung cancer. *Oncotarget*. 2014;5:10434-45.
15. Xie S, Li Y, Li X, Wang L, Yang N, Wang Y, et al. Mer receptor tyrosine kinase is frequently overexpressed in human non-small cell lung cancer, confirming resistance to erlotinib. *Oncotarget*. 2015;6:9206-19.
16. Papadakis ES, Cichon MA, Vyas JJ, Patel N, Ghali L, Cerio R, et al. Axl Promotes Cutaneous Squamous Cell Carcinoma Survival through Negative Regulation of Pro-Apoptotic Bcl-2 Family Members. *J Invest Dermatol*. 2011;131:509-17.
17. Zhu S, Wurdak H, Wang Y, Galkin A, Tao H, Li J, et al. A genomic screen identifies TYRO3 as a MITF regulator in melanoma. *Proceedings of the National Academy of Sciences*. 2009;106:17025-30.
18. EKYALONGO RC, MUKOHARA T, FUNAKOSHI Y, TOMIOKA H, KATAOKA Y, SHIMONO Y, et al. TYRO3 as a Potential Therapeutic Target in Breast Cancer. *Anticancer Research*. 2014;34:3337-45.
19. Lee C. Overexpression of Tyro3 receptor tyrosine kinase leads to the acquisition of taxol resistance in ovarian cancer cells. *Molecular Medicine Reports*. 2015;12.

20. Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet.* 2012;44:852-60.
21. Bae SY, Hong J-Y, Lee H-J, Park HJ, Lee SK. Targeting the degradation of AXL receptor tyrosine kinase to overcome resistance in gefitinib-resistant non-small cell lung cancer. *Oncotarget.* 2015;6:10146-60.
22. Meyer AS, Miller MA, Gertler FB, Lauffenburger DA. The Receptor AXL Diversifies EGFR Signaling and Limits the Response to EGFR-Targeted Inhibitors in Triple-Negative Breast Cancer Cells. *Science signaling.* 2013;6:ra66-ra.
23. Brand TM, Iida M, Stein AP, Corrigan KL, Braverman C, Luthar N, et al. AXL mediates resistance to cetuximab therapy. *Cancer research.* 2014;74:5152-64.
24. Vouri M, Croucher DR, Kennedy SP, An Q, Pilkington GJ, Hafizi S. Axl-EGFR receptor tyrosine kinase hetero-interaction provides EGFR with access to pro-invasive signalling in cancer cells. *Oncogenesis.* 2016;5:e266.
25. Paolino M, Penninger JM. The Role of TAM Family Receptors in Immune Cell Function: Implications for Cancer Therapy. *Cancers.* 2016;8:97.
26. Caraux A, Lu Q, Fernandez N, Riou S, Di Santo JP, Raulet DH, et al. Natural killer cell differentiation driven by Tyro3 receptor tyrosine kinases. *Nat Immunol.* 2006;7:747-54.
27. Paolino M, Choidas A, Wallner S, Pranjic B, Uribealago I, Loeser S, et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature.* 2014;507:508-12.
28. Kasikara C, Kumar S, Kimani S, Tsou WI, Geng K, Davra V, et al. Phosphatidylserine Sensing by TAM Receptors Regulates AKT-dependent Chemoresistance and PD-L1 Expression. *Mol Cancer Res.* 2017.
29. Wnuk-Lipinska K, Davidsen K, Blø M, Hodneland L, Engelsen A, Kang J, et al. Abstract B027: BGB324, a selective small molecule inhibitor of AXL receptor tyrosine kinase, enhances immune checkpoint inhibitor efficacy. *Cancer Immunology Research.* 2016;4:B027.
30. Buehler M, Tse B, Leboucq A, Jacob F, Caduff R, Fink D, et al. Meta-Analysis of Microarray Data Identifies GAS6 Expression as an Independent Predictor of Poor Survival in Ovarian Cancer. *BioMed Research International.* 2013;2013:9.
31. Nowlaczyk AU, Yu LG. Galectin-3--a jack-of-all-trades in cancer. *Cancer letters.* 2011;313:123-8.
32. Balaji K, Vijayaraghavan S, Diao L, Tong P, Fan Y, Carey JPW, et al. AXL Inhibition Suppresses the DNA Damage Response and Sensitizes Cells to PARP Inhibition in Multiple Cancers. *Molecular Cancer Research.* 2016.
33. Ben-Batalla I, Erdmann R, Jorgensen H, Mitchell R, Ernst T, von Amsberg G, et al. Axl Blockade by BGB324 Inhibits BCR-ABL Tyrosine Kinase Inhibitor-Sensitive and -Resistant Chronic Myeloid Leukemia. *Clin Cancer Res.* 2016.
34. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MBA, Lemke G. TAM Receptors Are Pleiotropic Inhibitors of the Innate Immune Response. *Cell.* 2007;131:1124-36.
35. Sharif MN, Šošić D, Rothlin CV, Kelly E, Lemke G, Olson EN, et al. Twist mediates suppression of inflammation by type I IFNs and Axl. *The Journal of Experimental Medicine.* 2006;203:1891-901.
36. Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet.* 2000;26:270-1.
37. Wallet MA, Sen P, Flores RR, Wang Y, Yi Z, Huang Y, et al. MerTK is required for apoptotic cell-induced T cell tolerance. *The Journal of experimental medicine.* 2008;205:219-32.

38. Bosurgi L, Bernink JH, Delgado Cuevas V, Gagliani N, Joannas L, Schmid ET, et al. Paradoxical role of the proto-oncogene Axl and Mer receptor tyrosine kinases in colon cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110:13091-6.
39. Lemke G. *Biology of the TAM Receptors*. Cold Spring Harbor Perspectives in Biology. 2013;5.