

TAM Receptor Tyrosine Kinases in Cancer Drug Resistance

Mikaella Vouri and Sassan Hafizi



Abstract

Receptor tyrosine kinases (RTK) 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 the 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. *Cancer Res*; 77(11); 2775–8. ©2017 AACR.

Introduction

The TAM (Tyro3, Axl, MerTK) family of receptor tyrosine kinases (RTK) 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 to 140 kDa for Axl and Tyro3 and 165 to 205 kDa for MerTK due to posttranslational 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; ref. 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 tumor 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 proinflammatory 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 expression with resistance to the small molecule inhibitor imatinib (targets BCR-Abl, c-Kit, and PDGFR) in chronic myelogenous leukemia (CML; ref. 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 cell lung carcinoma (NSCLC; ref. 9).

MerTK overexpression has been detected in high-grade glioblastomas and its knockdown to result in increased sensitivity to etoposide while, conversely, control cells showed elevated MerTK activation upon DNA damage with increase resistance to etoposide (10). Inhibition of Axl and MerTK also led 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; ref. 13). MerTK inhibition by an mAb was able to increase sensitivity to carboplatin in 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).

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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 proapoptotic Bcl-2 family proteins (16). Recently in melanoma, Tyro3 was shown to regulate the expression of microphthalmia-associated transcription factor, 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, down-regulation 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 led 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 (DC), and they may cooperate to create an immunosuppressive tumor microenvironment permissive to tumorigenesis (25). MerTK is implicated in the suppression of the M1 macrophage proinflammatory cytokine response, and Axl is required for the termination of Toll-like receptor-dependent inflammatory response in DCs (25). Also, the TAMs have been shown to negatively regulate natural killer (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, while treatment of wild-type NK cells with a TAM-selective small-molecule inhibitor enhanced antimetastatic 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, for example, 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 tumor clearance, survival, and tumor infiltration of cytotoxic T cells (29).

Other influences that can contribute further to TAM-mediated tumor chemoresistance could include increased expression of 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 Tumor 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 tumors. 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 tumor drug resistance.

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.

Table 1. TAM-selective inhibitors currently in development

Name	Target(s)	Development stage
DP3975	Axl	Preclinical
LDC1267	Axl, Tyro3, MerTK	Preclinical
NA80×1	Axl	Preclinical
YW327.6S2	Axl	Preclinical
GL21.T	Axl	Preclinical
NPS-1034	Axl, c-Met	Preclinical
UNC1062	MerTK	Preclinical
UNC569	MerTK	Preclinical
BGB324	Axl	Phase I clinical trial for NSCLC (NCT02922777), AML (NCT02488408), and metastatic melanoma (NCT02872259)
BMS777607	c-Met, Ron, Flt-3, Tyro3, MerTK, Axl	Phase I clinical trial for advanced and/or metastatic solid tumors (NCT01721148)
Glesatinib (MGCD265)	Axl, c-Met	Phase II clinical trial for NSCLC patients with activating mutations in <i>MET</i> (NCT02544633)
Sunitinib (SU1248)	Flt-3, Axl, VEGFR-2, Kit	Approved for pancreatic neuroendocrine and gastrointestinal stromal tumor and kidney cancer.
Cabozantinib	VEGFR-2, c-Met, Ret, Kit, Flt-3, Axl	Approved for medullary thyroid cancer

NOTE: All are small-molecule inhibitors except YW327.6S2, which is an mAb, and GL21.T, which is an RNA aptamer.

Abbreviation: AML, acute myeloid leukemia.

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 antitumor immunity. Both Axl and MerTK are expressed on multiple immune cells, such as DCs and macrophages and have an essential immunosuppressive 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 codependent on TAM receptors (35). In addition, 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 MerTK blockade has also been shown to result in a greater proportion of DCs with enhanced T-cell activation capacity versus tolerogenic DCs (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 tumor cells. Also, Axl and MerTK double knockout mice displayed enhanced colitis resulting from insufficient neutrophil clearance (25) and an increased production of proinflammatory cytokines favoring a tumor-promoting environment with enhanced colonic polyp 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 DCs 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 proinflammatory effects that may conversely favor tumor promotion as opposed to a desired antitumor 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 antitumor immunity specifically.

The TAM RTKs represent a distinct grouping of novel anticancer targets through their promotion of tumor cell survival, proliferation, invasion, and chemoresistance, as well as suppression of the immune status of the tumor 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 anticancer drug resistance for many solid and hematologic malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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