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TAM Receptors in Leukemia: Expression, Signaling, and Therapeutic Implications

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Abstract

In the past 30 years there has been remarkable progress in the treatment of leukemia and lymphoma. However, current treatments are largely ineffective against relapsed leukemia and, in the case of pediatric patients, are often associated with severe long-term toxicities. Thus, there continues to be a critical need for the development of effective biologically targeted therapies. The TAM family of receptor tyrosine kinases—Tyro3, Axl, and Mer—plays an important role in normal hematopoiesis, including natural killer cell maturation, macrophage function, and platelet activation and signaling. Furthermore, TAM receptor activation leads to upregulation of pro-survival and proliferation signaling pathways, and aberrant TAM receptor expression contributes to cancer development, including myeloid and lymphoid leukemia. This review summarizes the role of TAM receptors in leukemia. We outline TAM receptor expression patterns in different forms of leukemia, describe potential mechanisms leading to their overexpression, and delineate the signaling pathways downstream of receptor activation that have been implicated in leukemogenesis. Finally, we discuss the current research focused on inhibitors against these receptors in an effort to develop new therapeutic strategies for leukemia.

Keywords

leukemia; Axl; Mer; Tyro-3; signal transduction; gene expression; targeted; therapeutics

I. INTRODUCTION

Cancer is the second leading cause of death in the United States, resulting in an estimated 23.2% of all deaths in 2007. While leukemia and lymphoma only account for approximately 7.6% of these deaths (43,370 in 2010), leukemia is the primary cause of cancer-related death in males under 40 and females under 20 years old. Furthermore, cancer is the leading cause of disease-related deaths in children (1–14 years old), and leukemia is the most common pediatric malignancy.^{1–3}

Between 1975 and 2003, the 5-year relative survival for all leukemia patients has increased significantly from 33.4% to 55.6%. This trend is even more dramatic for children with leukemia, where relative survival rates have increased from 43% to 94.6% in cases of acute

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lymphoblastic leukemia (ALL) and from 18.7% to 56.1% in cases of acute myeloid leukemia (AML).³ Although much of this improvement stems from changes in treatment regimens and dosing during induction and subsequent courses of therapy, the current success is associated with a 2- to 4-fold increased rate of severe therapy-associated health conditions, including organ damage, infertility, growth decline, reduced mental function, and secondary malignancy.^{4,5} Furthermore, current treatment protocols are largely ineffective against relapsed leukemia,⁶ highlighting the need for new antileukemic therapeutic agents.

The TAM family of receptor tyrosine kinases (RTKs) includes Tyro-3, Axl, and Mer. In normal hematopoiesis, TAM receptors inhibit inflammation in dendritic cells and macrophages, promote phagocytosis of apoptotic cells and membranous organelles, and are essential for natural killer (NK) cell maturation.⁷ The TAM family is also vital for platelet activation, platelet signaling during thrombus stabilization,^{8,9} and may have an important role in erythropoiesis.¹⁰ In addition to their role in normal hematopoiesis, TAM receptors can activate proliferation- and survival-promoting signaling pathways such as those driven by AKT and ERK1/2, which contribute to oncogenesis in multiple cancers, including myeloid and lymphoid leukemia.¹¹

The goal of this review is to summarize the role of the TAM family in leukemia. Because little published data exist on Tyro-3 in hematopoietic malignancy, we have largely focused on Mer and Axl receptor tyrosine kinases. Throughout the discussion, we address leukemia-associated expression patterns observed for TAM receptors, explore potential mechanisms underlying their aberrant expression, and outline the downstream signaling pathways implicated in leukemogenesis. In the final part of our review, we detail the ongoing efforts to develop therapeutic agents against this family of receptors and discuss how specifically targeting TAM receptors can potentially enhance leukemia therapy.

II. TAM RECEPTOR EXPRESSION PATTERNS IN LEUKEMIA

While macrophages, dendritic cells, NK cells, NKT cells, megakaryocytes, and platelets normally express TAM receptors,^{7,8,10,12,13} they are not expressed in thymocytes, mature T- or B-lymphocytes, or granulocytes.¹⁴ However, TAM receptors display altered expression patterns in leukemia. Below and in Table 1, we compare TAM receptor expression in different subsets of leukemia.

A. Tyro-3

Tyro-3 (Dtk/Sky/Rse/Br/tif) RNA was identified in blasts in 6 of 11 AML patients by RNase protection analysis.¹⁵ More recently, a gene expression microarray found Tyro-3 overexpression in multiple myeloma samples relative to autologous B-lymphoblastoid cell lines, and Tyro-3 mRNA transcript was also detected in primary malignant plasma cells from patients with plasma cell leukemia or multiple myeloma.¹⁶

B. Axl

Axl (Ufo/Jtk11) was first detected in 1988 as an unidentified gene promoting the transition from chronic phase to blast crisis in two patients with chronic myelogenous leukemia (CML).¹⁷ Three years later, two independent groups cloned the human gene from patients

with CML and chronic myeloproliferative disorder.^{18,19} Axl mRNA expression was identified in a large number of cell lines derived from myeloid and erythromegakaryocytic leukemias and was notably absent in lymphocytic cell lines.²⁰ Patient sample analysis confirmed these cell line results, revealing Axl transcript in 56/99 (56.6%) patients with myeloproliferative disorders (AML, CML in chronic phase and in blast crisis, and myelodysplasia), but in only 2.2% of lymphoid leukemias: out of 45 samples, only one patient with chronic lymphocytic leukemia (CLL) had detectable Axl mRNA.^{21,22} Similarly, Rochlitz et al. identified Axl transcript in 19 of 54 (35%) AML patient samples, observing that patients with increased CD34 expression also displayed higher levels of Axl, and they determined that Axl expression was associated with worse progression-free and overall survival when adjusted for age, Auer rods, and leukocyte counts.²³ Lastly, a recent study found increased Axl transcript in myeloid leukemia samples from chemotherapy-resistant patients; furthermore, chemotherapy induced Axl expression in an AML cell line, and addition of Gas6—an Axl ligand—enhanced drug resistance in cells during chemotherapeutic treatment.²⁴

C. Mer

The human Mer gene (MerTK/RP38/Nyk/Tyro12) was initially cloned from a B-lymphoblastoid expression library.¹⁴ While normal T- and B-lymphocytes do not express Mer mRNA transcript or protein at any developmental stage,^{25,26} our lab has detected ectopic Mer mRNA expression in 19/34 (55.8%) patient samples, and Mer protein expression in 8/16 (50%) pediatric T-ALL patient samples.²⁵ Furthermore, a large-scale microarray analysis demonstrated that significantly high levels of Mer transcript exist in the cytogenetic subset of B-ALL patients expressing the *E2A-PBX1* fusion protein.²⁷ Consistently, our lab has identified aberrant Mer protein expression in 16/16 *E2A-PBX1* B-ALL patient samples, whereas 11/12 B-ALL samples without *E2A-PBX1* did not express Mer.²⁶ To date, there are no published reports on the role of Mer in myeloid leukemia, but we have detected increased Mer expression in 11/16 AML cell lines and in 17/26 primary patient samples by western blot and flow cytometry.²⁸

The role of Mer in leukemogenesis is further supported by two animal models. Abnormal activation of Eyk, the chicken homologue of Mer, via the naturally occurring RPL30 avian retrovirus, leads to the development of a spectrum of cancers, including lymphomas, in chickens.²⁹ Additionally, ectopic Mer expression in lymphocytes in the Mer transgenic mouse increases the incidence of leukemia/lymphoma.³⁰

III. UPSTREAM REGULATION OF TAM RECEPTOR EXPRESSION

TAM receptor overexpression occurs in many cancers of myeloid lineage, and ectopic expression of Mer, which normal lymphocytes do not express, is found in mantle cell lymphoma, the majority of T cell leukemias, and particular subsets of B cell leukemia.^{25,27} Although aberrant TAM receptor levels clearly enhance oncogenic potential, much remains unknown about the mechanisms underlying their overexpression. Several studies have begun to explore epigenetic and post-transcriptional regulation of TAM receptor expression, providing us with further insight into the tangled circuitry of cancer progression. Although

the studies presented here have been conducted in a variety of systems, their findings may also apply to processes within hematopoietic development and leukemogenesis, which are depicted in Figure 1.

A. Genetic Variation

To date, no activating mutations in the TAM receptor genes have been implicated in malignant transformation, but recent studies have highlighted the potential role of copy number variation in TAM receptor expression. *AXL* gene amplification and corresponding overexpression of its transcript were found in a CGH-based microarray profiling study of glioblastoma samples,³¹ and gastric cancer samples displayed increased *AXL* and *MER* copy numbers relative to normal controls.³² Additionally, DNA copy number analysis identified *AXL* gene amplification in 4/4 lapatinib-resistant breast cancer cell lines,³³ and *GAS6* amplification has also been detected in aggressive mouse mammary tumors.³⁴

Analysis of the Axl transcript has also shown that two alternatively spliced isoforms are expressed in tumor and normal samples at different ratios. However, both isoforms have the same transforming capability, suggesting that receptor overexpression—rather than a structural difference in the transcript or protein—drives the oncogenicity of this receptor.¹⁸

B. Transcriptional Regulation

While several putative transcription factors for the TAM receptor genes have been identified based on promoter binding site specificity, gene expression modulation has been most extensively studied in Axl, as it is the only human TAM receptor for which the gene promoter has been fully characterized. Multiple studies have found that AP-2α, Sp1/Sp3 and MZF-1 directly regulate Axl transcription, with MZF-1 levels directly correlating with Axl expression and metastasis in colorectal and cervical cancers.^{18,35–37} More recently, CXCR4/SDF-1 (CXCL12) has been shown to increase transcription of both *AXL* and *TYRO3* in thyroid carcinoma cell lines; although the transcriptional interaction was not further characterized, treatment with a CXCR4 inhibitor did not reduce constitutive Axl expression, suggesting that its overexpression requires additional regulatory mechanisms.³⁸ Another study found that Gas6, the common ligand for both Axl and Mer, was transcriptionally upregulated following progesterone receptor activation in breast cancer cells.³⁹ A complete list of transcription factors and their interactions with the TAM receptor genes has been compiled in Table 2.

Although the human Mer promoter remains completely uncharacterized, Wong et al. identified Sp1, Sp3, and E2F as transcriptional regulators of the mouse *Mertk* gene,⁴⁰ which shares considerable homology with its human counterpart. Liver X receptor (LXR) was recently shown to directly bind the *Mertk* promoter and induce its transcription in mouse macrophages (without noticeable effects on Axl or Tyro-3 expression), which promoted phagocytosis of apoptotic cells and maintained immunity.⁴¹ Another study found that CLL cells display increased LXR expression relative to normal lymphocytes, but the potential relationship with Mer expression was not investigated.⁴² However, disruption of normal LXR expression in lymphocytes causes age-dependent lymphoid hyperplasia in mouse

models,⁴³ findings similar to those observed upon ectopic Mer expression in transgenic mouse lymphocytes.³⁰

Mer has also been identified as a glucocorticoid-responsive gene in mouse mesenchymal stem-like cells: dexamethasone treatment led to a 4.6-fold increase in Mer expression relative to vehicle-treated cells, and ChIP-chip analyses revealed several glucocorticoid binding sequences upstream of the *Mertk* transcription start site.⁴⁴ Another study found a similar trend in dexamethasone-induced Mer protein expression on the surface of cultured human macrophages,⁴⁵ indicating that Mer upregulation may decrease responsiveness to ALL induction therapy, which involves glucocorticoid treatment. Consistently, our laboratory has found increased Mer expression in patients with relapsed leukemia (Eisenman K and Graham DK, unpublished data), suggesting that Mer may serve as a survival or resistance mechanism in leukemic cells following treatment.

Although Mer is upregulated in the *E2A-PBX1*+ cytogenetic subset of B-ALL patients,^{26,27,46} expression of the fusion protein does not appear to induce Mer transcription (Sawczyn K and Graham DK, unpublished data) despite the presence of a PBX1-binding site near the Mer promoter.⁴⁷ However, the PBX domain of the fusion protein is directly responsible for inducing apoptosis in hematopoietic precursor B cell lines,⁴⁸ consistent with the idea that Mer expression arises as a survival response rather than directly through E2A-PBX1-driven changes in gene transcription.

C. Epigenetic Regulation

Upon identifying Axl as the only consistently upregulated protein tyrosine kinase in drug-resistant AML patient samples, Hong et al. found that treatment of an AML cell line with a chemotherapeutic agent—doxorubicin, cisplatin, or VP16—increased Axl expression, but only when its promoter remained unmethylated.²⁴ Furthermore, a separate study correlated promoter hypomethylation with the degree of Axl overexpression in Kaposi sarcoma: cell lines expressing high levels of Axl had fewer methylated sites, whereas other lines expressing less Axl—including some derived from other cancer types—displayed more promoter methylation.⁴⁹ Lastly, it was shown that SAHA, a histone deacetylase (HDAC) inhibitor, suppresses Gas6 expression in multiple myeloma cells,⁵⁰ highlighting another potential mechanism regulating transcription of TAM receptor-related genes.

D. Post-Transcriptional Regulation

Currently, miR-335 is the only microRNA reported to target the Mer 3'UTR.⁵¹ However, the study, which compared expression differences between metastatic and non-metastatic breast cancer lines, only used indirect target-validation methods and never assessed Mer protein expression following manipulation of miR-335 levels. In functional studies of miRNAs in leukemic cells, restoration of miR-335 expression levels with a synthetic mimic did not decrease Mer protein expression (Migdall-Wilson J and Graham DK, unpublished data).

Both miR-155 and miR-34a decrease Axl expression in human monocytes.⁵² miR-155, which promotes tumor growth in numerous leukemias and lymphomas when overexpressed,⁵³ represses translation of several transcription factors that potentially regulate Axl and Mer expression, including PU.1, CEBP β , CSF1R, and HIF-1 α . In addition

to showing that miR-34a and miR-199a/b target the Axl 3'UTR, a recent study also determined that both miRNA genes and *AXL* are regulated by promoter ethylation.⁵⁴

Evdokimova et al. found that YB-1, a regulatory RNA-binding protein, normally inhibits translation of Mer mRNA; however, in conditions requiring increased expression of stress- and growth-related proteins, Mer translation is de-repressed upon AKT-mediated phosphorylation.⁵⁵ While this mechanism has not been explored in hematopoietic cells, the fact that AKT is a well-known downstream target of Mer raises the possibility of a positive-feedback loop occurring through this post-transcriptional mechanism.

IV. MER AND AXL RECEPTOR SIGNALING IN LEUKEMIA

Mer and Axl activate many different signaling pathways depending on cell type and function, a topic extensively discussed in a review previously published by our lab.¹¹ In this section, we specifically focus on the Mer- and Axl-activated pathways known to play a role in leukemogenesis (Figure 2).

A. Ligands

Gas6 and Protein S are two structurally similar, vitamin K-dependent proteins that activate the TAM receptors.^{56,57} Both ligands are produced in a wide range of tissues, including the bone marrow, thymus, spleen, and plasma,^{56,58,59} suggesting that leukemia cells expressing Mer or Axl are constitutively activated through a paracrine mechanism. Consistent with this idea, Gas6 expression has been correlated with the ability of bone marrow stromal cell lines to support hematopoiesis,⁶⁰ and primary human osteoblasts have been shown to secrete Gas6 in response to Mer-expressing leukemic cells.⁶¹ Furthermore, overexpression of Gas6 and Protein S has been reported in several cancers and often correlates with poor prognostic markers.⁶²

Tubby and Tubby-like protein 1 (Tulp1), which also bind Axl and Tyro-3, were recently described as two new Mer ligands important in phagocytosis.⁶³ It is unclear whether these two ligands play a role in leukemogenesis.

B. MAPK (ERK) Pathway

Before the TAM receptor ligands had been identified, initial studies on Mer and Axl downstream signaling implemented chimeric receptors^{64–66} composed of the Axl or Mer intracellular kinase domain fused to an extracellular domain of a receptor with a known ligand. In a study using a colony stimulating factor 1 (CSF1) receptor/Mer chimeric receptor, stimulation with CSF1 led to phosphorylation of Shc, recruitment of Grb2 and downstream phosphorylation of MEK and ERK1/2 kinases, indicating MAPK pathway activation.⁶⁶ These results were later confirmed with a CD8/Mer chimeric receptor in BaF3 cells,⁶⁵ and eventually in experiments using the full-length Mer receptor and recombinant Gas6 for activation of the receptor in 293 cells.⁶⁷ More recently, our lab demonstrated that Mer expression mediates chemotherapy-induced activation of ERK1/2 in the 697 (human B-ALL) cell line,²⁶ and related studies showed that Axl stimulation activates the ERK1/2 kinases through Shc, Grb2, and Ras.^{64,68} Interestingly, results of other investigations have

suggested that Axl-mediated ERK1/2 activation is specifically required to induce proliferation and depends on PI3K/AKT and Src activity.^{69,70}

C. PI3K/AKT/mTOR

Many studies exploring downstream activation of the MAPK pathway have also identified the PI3K/AKT kinase cascade as an important component of Axl/Mer signaling.^{64,66,67,69} Several groups showed that the p85 subunit of PI3K binds to both Mer and Axl, and determined that this interaction requires both ligand binding and kinase activity.^{64,68,71}

In response to Axl stimulation, PI3K/AKT pathway activation is important for both cell proliferation—via ERK1/2 activation—as well as survival, which occurs through phosphorylation of Bad, a Bcl-2 family member;^{69,70} furthermore, this antiapoptotic effect appears to be independent of MAPK activity.⁶⁹ Interestingly, studies investigating how Mer stimulation affects the PI3K/AKT pathway were less conclusive, partly because they were performed in different cell types: in the Ba/F3 lymphoid cell line, Mer-mediated PI3K activation increased cell survival upon IL-3 withdrawal and minimally impacted cell proliferation.⁶⁵ However, in the 32D monocytic cell line, Mer-mediated PI3K activation alone did not sufficiently prevent apoptosis; instead, investigators found that the MAPK and PI3K pathways play parallel roles: inhibition of both cascades was necessary to elicit growth factor withdrawal-induced cell death.⁷¹

mTOR kinase, a key regulator of protein synthesis and cell growth, is one of the downstream effectors of the PI3K pathway. Goruppi et al. showed that rapamycin, an mTOR inhibitor, blocked Axl-mediated proliferation and survival in NIH 3T3 cells.⁶⁹ Furthermore, S6 kinase (S6K)—a downstream target of mTOR—is activated upon stimulation of a Mer chimeric receptor in NIH/3T3 cells.⁶⁶

D. NF-κB

The NF-κB family of transcription factors is a key regulator of cell growth, development, and survival. Aberrant NF-κB activation has been observed in various forms of leukemia/lymphoma⁷² and has also been implicated downstream of Mer and Axl/Gas6 signaling.^{65,73,74} Luciferase reporter assays revealed a 10-fold increase in NF-κB transcriptional activity in Ba/F3 cells expressing a constitutively active chimeric Mer receptor, along with more robust proliferation than vector-only control cells.⁶⁵ In NIH/3T3 cells, Gas6 induced a transient decrease in the level of IκB, which binds to and inhibits NF-κB, resulting in enhanced NF-κB DNA-binding activity and an NF-κB-dependent increase in expression of Bcl-xL, an anti-apoptotic protein.⁷³ Likewise, expression of a dominant negative IκB—which keeps NF-κB in a permanently bound, inactive state—inhibited Gas6-mediated survival, further implying that the anti-apoptotic effects downstream of TAM receptor activation require NF-κB activity.⁷³ Lastly, NF-κB activation depends upon PI3K/AKT activation, suggesting that extensive cross-talk exists between the various pathways downstream of Mer or Axl.^{65,73}

E. Jak/STAT Pathway

Constitutive activation of STAT proteins has been observed both in AML and ALL, as well as in a variety of other tumors (reviewed in Benekli et al.⁷⁵). Jak/STAT activation arises through various mechanisms, including cytokine overexpression and autocrine/paracrine stimulation,⁷⁶ as well as expression of a TEL-JAK2 fusion protein.⁷⁷

Both constitutive Jak activation and STAT phosphorylation have been associated with Mer and Axl activity. COS cells expressing a constitutively active chicken Mer receptor (CD8-Eyk chimera) displayed constitutive activation of STAT1, STAT3, and Jak1; the CD8-Eyk chimera also co-immunoprecipitated with Jak1.⁷⁸ More recently, Gas6-induced Axl stimulation led to STAT1 activation in mouse bone marrow dendritic cells, an effect dependent upon type I interferon (IFN) receptor expression.⁷⁹ Interestingly, Axl co-immunoprecipitated with the IFN receptor R1 chain, suggesting a possible mechanism for TAM receptor activation of the Jak/STAT pathway.

V. THERAPEUTIC TARGETING

Several novel biologically targeted therapies have shown great promise in improving therapeutic outcomes in leukemia. The most striking examples are imatinib, dasatinib, and nilotinib, tyrosine kinase inhibitors used to treat CML and Philadelphia chromosome-positive (Ph+) ALL.⁸⁰ Imatinib therapy resulted in a 73.8% rate of complete cytogenetic response after 19 months, compared with 8.5% for patients on earlier standards of care.⁸¹ Other examples include lestaurtinib, a FLT3 tyrosine kinase inhibitor currently in clinical trials for treatment of MLL-rearranged ALL, and rituximab, a monoclonal antibody against CD20 currently used in therapy for CD20+ leukemia/lymphoma.^{82,83}

Mer and Axl represent two novel targets for the development of new anti-leukemia therapies, and multiple studies of receptor inhibition highlight their potential within this realm. Mer inhibition significantly delays leukemia progression in a human ALL cell line xenograft (Brandão and Graham, unpublished data), and Axl inhibition reduces growth of lung and breast cancer xenograft tumors in immuno compromised mice.⁸⁴ Furthermore, inhibition of Mer dampens pro-survival pathway activation in a human B-ALL cell line and renders it more sensitive to a spectrum of chemotherapeutic agents currently used in the clinic.²⁶ Similarly, Mer and Axl inhibition also increases apoptosis and chemosensitivity in astrocytoma cell lines.⁸⁵

Three different strategies are available to hinder TAM receptor signaling: ligand sinks, antibody-mediated downregulation/blocking, and small-molecule kinase inhibitors. Our lab has shown that treatment with a Mer-Fc fusion construct—one example of a ligand sink—inhibits Gas6 signaling, apoptotic cell engulfment by macrophages, and platelet aggregation.⁸⁶ Such a construct could be used as adjuvant therapy to transiently inhibit TAM signaling in leukemia cells at the time of chemotherapy administration.

Two separate groups have reported that Axl-specific antibodies inhibit signaling and enhance the effect of standard chemotherapies on tumor cells.^{84,87,88} Zhang et al. showed that treatment with a polyclonal anti-Axl antibody significantly diminishes the motility and

invasive properties of human breast cancer cells.⁸⁸ Furthermore, Ye et al. demonstrated that an anti-Axl monoclonal antibody blocks Gas6 signaling, reduces Axl surface expression, and synergizes with standard chemotherapy agents to inhibit tumor growth in xenograft models of lung and breast cancer.⁸⁷ While there are no published studies regarding the use of Mer-specific antibodies as a therapeutic agent, our lab has characterized monoclonal anti-Mer antibodies that decrease surface expression of Mer in human ALL cell lines.

Although there are few reports of TAM receptor-specific small-molecule inhibitors, several inhibitors developed against closely related receptor tyrosine kinases,⁸⁹ such as c-Kit and Met, are also active against Axl and/or Mer. Foretinib (GSK1363089, GlaxoSmithKline), a Met inhibitor currently in several clinical trials for use against solid tumors, inhibits *in vitro* Axl phosphorylation in the low nanomolar range ($IC_{50}=11$ nM).³³ MP470 (Amuvatinib, SuperGen), originally designed as a c-Kit inhibitor, inhibits Axl activation^{90,91} and is currently in a Phase II clinical trial to test it as combination therapy with Platinum-Etoposide against small cell lung carcinoma. Crizotinib (PF-2341066, Pfizer), a Met inhibitor also active against ALK kinase, inhibits Axl with an $IC_{50}=322$ nM,⁹² and is currently being evaluated in a variety of clinical trials. Lastly, BMS777607 (Bristol-Meyers Squibb)—also developed against Met—has low nanomolar activity *in vitro* against Axl, Mer and Tyro-3 ($IC_{50}=1.1, 14,$ and 4.3 nM, respectively) and has been evaluated in a Phase I dose-escalation clinical trial.⁹³

Unlike the inhibitors mentioned above, R428 (Rigel) was developed specifically against Axl.⁹⁴ Treatment with R428, which inhibits Axl with an $IC_{50}=14$ nM but shows low activity for Mer and Tyro3, blocked both *in vitro* invasion and *in vivo* metastatic potential of a human breast cancer cell line, and also prolonged survival in a mouse model of post-mastectomy metastasis.⁹⁴ In a separate study, R428 was also shown to induce apoptosis in CLL B cells.⁹⁵

In contrast with Axl, there is no information on specific small molecule inhibitors targeting Mer or Tyro-3. In a crystal structural study of Mer, a library of kinase inhibitors was screened *in vitro* against the kinase domain of the receptor⁹⁶; several compounds were identified, but no further reports of biological activity or toxicity are available.

VI. CONCLUSION

In this review, we have described the role of the TAM receptor family in leukemia to spotlight the aberrant expression patterns within different subsets and illustrate how receptor activation upregulates particular proliferative and antiapoptotic signaling pathways known to contribute to leukemogenesis. Although the mechanisms underlying abnormal TAM receptor expression are still unclear, the existing data—insight gained from studies conducted on various cell types—underscore how multiple, and perhaps context-dependent, processes facilitate overexpression of TAM receptors in leukemic cells.

The preclinical data presented here strongly support the development of specific inhibitors of TAM receptors: Mer and/or Axl inhibition can enhance leukemia cell sensitivity to chemotherapeutic agents currently used in the clinic, thus requiring lower doses to achieve

equal or better efficiency and decreasing the severity of side effects observed with current therapies. Given that patients with inactivating Mer mutations and TAM receptor-knockout mice experience late-onset health defects,^{97–101} transient TAM inhibition during chemotherapy will not likely bear the same long-term consequences. Furthermore, current immunophenotyping assays can easily be adapted to characterize TAM expression in leukemia patient samples,^{25,28} highlighting the diagnostic value of TAM family receptors. For all of these reasons, we believe that incorporating TAM receptor-targeted therapies into treatment regimens for leukemia, as well as for other cancers, is an exciting strategy in the ongoing fight against this devastating disease.

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ABBREVIATIONS

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
CLL	chronic lymphocytic leukemia
CML	chronic myelogenous leukemia
CSF1	colony stimulating factor 1
HDAC	histone deacetylase
IFN	type I interferon
LXR	liver X receptor
NK	natural killer
NKT	natural killer T cell
RTK	receptor tyrosine kinase
TAM	Tyro3, Axl, and Mer

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FIGURE 1.

Experimentally determined regulators of TAM receptor and ligand gene expression. Nuclear modulators include transcription factors, histone acetylation, promoter methylation, and gene amplification. Outside of the nucleus, several post-transcriptional processes influence protein formation: miRNAs repress translation of Axl and HIF-1α, potentially altering both *MER* and *AXL* transcription; YB-1, an RNA-binding protein, inhibits Mer translation unless it is phosphorylated by AKT, a downstream target of Mer activation.

FIGURE 2.

Mer/Axl signaling in leukemia. Receptor activation by Gas6 or Protein S leads to activation of MAPK, PI3K/AKT and Jak/STAT pathways, resulting in increased proliferation and survival. Jak/STAT activation has been observed both via direct interaction between Jak and Mer, as well as through interaction between Axl and the type 1 interferon receptor (IFNAR₁).

TABLE 1

Expression of TAM Receptors in Leukemia and in the Hematopoietic System

Leukemia	Tyro-3	Axl	Mer	Gas6	Protein S	References
<i>Myeloid</i>						
CML		Pos	Pos	Pos		17, 18, 21, 102
AML	Pos	Pos	Pos	Pos		15, 20, 21, 23, 28, 102
Myeloblastic leukemia		Pos	Pos	Pos		20, 28, 102
Monoblastic leukemia		Pos	Pos	Pos		20, 28, 102
Erythroid leukemia		Pos	Pos	Pos		20, 28, 102
Megakaryoblastic leukemia		Pos	Pos	Pos		20, 28, 102
<i>Lymphoblastic</i>						
ALL		Neg	Pos	Pos	Pos	14, 20, 21, 25–27
CLL		Pos		Pos		21, 95, 102
Plasma cell leukemia	Pos					16
<i>Normal hematopoiesis</i>						
Lymphocytes		Neg	Neg			14, 20, 21
Granulocytes		Neg				21
Basophil/Mast cells		Pos				7
Macrophages	Pos	Pos	Pos	Pos	Pos	7, 13, 14, 21, 103
Megakaryocytes/platelets	Pos	Pos	Pos	Pos		7, 14, 104–106
NK cells, NKT cells	Pos	Pos	Pos	Neg	Pos	7, 13
Dendritic cells	Pos	Pos	Pos	Pos	Pos	7, 13
Bone marrow	Neg	Pos	Pos	Pos		14, 22, 106, 107
Erythroid cells	Neg	Pos	Pos	Pos		10, 108

TABLE 2

Transcriptional Regulators of TAM Receptor Expression

Gene	Interaction	Transcription Factor	Effect	Cell Type	Reference
Tyro-3	Indirect	NGF	↑	Rat neuronal cells	109
Tyro-3	Indirect	CXCR4/SDF-1 (CXCL12)	↑	Thyroid carcinoma	38
Axl	Direct	AP-2a	↑	HeLa, 293T, NIH3T3, NSC-34	37
Axl	Direct	Sp1/Sp3	↑	Rko, HCT116, HeLa	36
Axl	Direct	MZF-1	↑	Colorectal, cervical cancer	110
Axl	Indirect	CXCR4/SDF-1 (CXCL12)	↑	Thyroid carcinoma	38
Axl	Indirect	HIF-1a	↑	Endothelial cells	111
Axl	Indirect	Net/Elk3	↑	Endothelial cells	112
Axl	Indirect	NGF	↑	Rat neuronal cells	109
Axl	Indirect	Prox-1	↓	Blood endothelial cells	113
Axl	Indirect	E1A	↓	Breast cancer	35
Axl	Predicted	AP-1			24
Axl	Predicted	C/EBPb			24
Axl	Predicted	p300			24
Axl	Predicted	CREB			24
Mer	Direct	Sp1	↑, ↓	Mouse Sertoli cells	40
Mer	Direct	LXR	↑	Macrophages	41
Mer	Direct	Sp3	↑	Mouse Sertoli cells	40
Mer	Direct	E2F	↓	Mouse Sertoli cells	40
Mer	Indirect	BRLF1 (R)	↑	Several	114
Mer	Indirect	PAX-FKHR	↑	Mouse MSCs	115
Mer	Indirect	GR	↑	Mouse MSCs	44
Mer	Indirect	Net/Elk3	↓	Endothelial cells	112
Mer	Indirect	HIF-1a	↓	Endothelial cells	112
Mer	Predicted	GATA			40
Mer	Predicted	MZF-1			40
Gas6	Direct	ER-a	↑	Breast cancer, normal mammary	116
Gas6	Indirect	PR-B	↑	Breast cancer	39