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Mertk: An emerging target in cancer biology and immuno-oncology

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Abstract

Mertk, a type I Receptor Tyrosine Kinase (RTK) and member of the TAM (Tyro3, Axl, and Mertk) family of homologous tyrosine kinases, has important roles in signal transduction both homeostatically on normal cells as well as patho-physiologically on both tumor-associated macrophages and malignant cells by its overexpression in a wide array of cancers. The main ligands of Mertk are Vitamin K-modified endogenous proteins Gas6 and Protein S (ProS1), heterobifunctional modular proteins that bind Mertk via two carboxyl-terminal laminin-like globular (LG) domains, and an N-terminal Gla domain that binds anionic phospholipids, whereby externalized phosphatidylserine (PS) on stressed viable and caspase-activated apoptotic cells is most emblematic. Recent studies indicate that Vitamin K-dependent γ-carboxylation on the N-terminal Gla domain of Gas6 and Protein S is necessary for PS binding and Mertk activation, implying that Mertk is preferentially active in tissues where there is high externalized PS, such as the tumor microenvironment (TME) and acute virally infected tissues. Once stimulated, activated Mertk can provide a survival advantage for cancer cells as well as drive compensatory proliferation. On monocytes and tumor-associated macrophages, Mertk promotes efferocytosis and acts as an inhibitory receptor that impairs host anti-tumor immunity, functioning akin to a myeloid checkpoint inhibitor. In recent years, inhibition of Mertk has been implicated in a dual role to enhance the sensitivity of cancer cells to cytotoxic agents along with improving host anti-tumor immunity with anti-PD-1/PD-L1 immunotherapy. Here, we examine the rationale of Mertk-targeted immunotherapies, the current and potential therapeutic strategies, the clinical status of Mertk-specific therapies, and potential challenges and obstacles for Mertk-focused therapies.

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1. Introduction

1.1 Discovery and expression of Mertk

Mertk, first discovered in 1992 by Hanafusa and colleagues, was initially isolated as a truncated tyrosine kinase (Env-Ryk, v-Ryk, v-Eyk) from the transforming gene of chicken retrovirus RPL30 (Jia et al., 1992). Subsequent cloning of the cellular gene identified Eyk (c-Eyk) as a novel type I receptor tyrosine kinase with homology to UFO/Axl, containing an N-terminal extracellular domain, a single transmembrane domain, and a C-terminal intracellular domain (Jia and Hanafusa, 1994). By domain homology, the extracellular portion contains two ligand-binding immunoglobulin-like C2-type (Ig) domains and two fibronectin-type III (FNIII) domains while the intracellular portion contains a tyrosine kinase signaling domain. C-Eyk was later named c-Mer (Mertk) by Graham and colleagues following cloning from a human B lymphoma expression library and aptly named as it was predominantly expressed on myeloid cells, epithelial cells, and cells of the reproductive tract (Graham et al., 1995). However, recent studies have shown that Mertk is likely more broadly expressed in a wider variety of cells that include macrophages, NK cells, dendritic cells (Behrens et al., 2003), T cells (Cabezon et al., 2015; Peeters et al., 2019), platelets (Zhou et al., 2018), spermatogonia (Wang et al., 2005), Sertoli cells (Wang et al., 2005), retinal pigment epithelium (D'Cruz et al., 2000; Nandrot et al., 2000), and oocytes (Kim et al., 2010). Patho-physiologically, overexpression of Mertk has been observed in B and T cell leukemias, including T-cell acute lymphoblastic leukemia and aggressive B cell lymphoma (Keating et al., 2006), as well as on a growing list of solid cancers, including gastric cancer (Yi et al., 2017), non-small cell lung cancer (Xie et al., 2015), colon cancer (Bosurgi et al., 2013), prostate cancer (Cackowski et al., 2017), and breast cancer (Davra et al., 2020; Kasikara et al., 2017; Nguyen et al., 2014; Png et al., 2011; Wu et al., 2017).

1.2 Mertk ligands: Gas6 and Protein S

The physiological and most canonical ligands for Mertk are Gas6 and Protein S, homologous proteins that contain an N-terminal Vitamin K-dependent γ-carboxyglutamic acid (GLA) domain, four epidermal growth factor-like (EGF-like) domains with homology to tissue factor, and two carboxy-terminal receptor binding laminin G-like (LG) domains (Chen et al., 1997; Hall et al., 2005; Manfioletti et al., 1993; Mark et al., 1996; Nagata et al., 1996; Sugo et al., 1986) (Fig. 1). By virtue of post-translationally modified γ-carboxyglutamic acid residues in the Gla domain, the Gla domain gains the ability to interact with calcium ions that permits interaction with the negatively charged phosphatidylserine (PS) headgroup on the surface of apoptotic cells and viable stressed cells, effectively bridging externalized PS to Mertk (Nakano et al., 1996, 1997; Sugo et al., 1986). Importantly, by mechanisms that are still not well understood, γ-carboxylation of the Gla domain of Gas6 and ProS1 is absolutely required to produce biologically active ligand, as ligand generated in the presence of Warfarin, an inhibitor of Vitamin K-dependent carboxylase (VKORC1) and Gas6 γ-carboxylation, produces a non-functional ligand that cannot stimulate Mertk activation and subsequent tyrosine phosphorylation (Tsou et al., 2014). Interestingly, inactive/non-carboxylated Gas6 and Protein S are still capable of binding to Mertk receptors and are expected to compete with active/γ-carboxylated ligands,

providing the rationale, as described below, to produce non-γ-carboxylated Gas6 proteins as dominant-negative functional receptor traps and novel TAM therapeutics (Tsou et al., 2014).

1.3 Role of phosphatidylserine in Mertk activation

In addition to the requirement of functional γ-carboxylation of Gas6 and ProS1 to produce active ligands and stimulate Mertk, PS sources such as exogenous PS liposomes, apoptotic cells, Ca^{2+}-stressed viable cells, or PS-positive exosomes all significantly amplify tyrosine phosphorylation and post-receptor signaling compared to Gas6 without PS opsonization (Eken et al., 2010; Tsou et al., 2014; Uehara and Shacter, 2008) (Fig. 1). Importantly, such observations suggest that Mertk signaling will be robust and constitutive in areas with active ligand present and elevated levels of externalized PS. Due to the increased levels of metabolic stress and apoptosis, high PS levels are present in many solid tumors (Gerber et al., 2011; He et al., 2009; Jennewein et al., 2008; Ran and Thorpe, 2002). Likewise, Gas6 overexpression is present and well-documented in numerous cancer types including melanoma, lung cancer, gastric cancer, prostate cancer, and breast cancer where it promotes cancer progression and is associated with poorer prognoses (Dirks et al., 1999; Hutterer et al., 2008; Lee et al., 2013; Mc Cormack et al., 2008; Sainaghi et al., 2005; Sun et al., 2003, 2004; Wu et al., 2017). Therefore, the tumor microenvironment (TME) offers a unique niche in which Mertk, expressed on the cancer itself or on tumor-associated macrophages, is constitutively active and amplified.

2. Consequences of Mertk activation

2.1 Post-receptor signaling

Since Mertk is expressed on multiple cell types, its post-receptor signaling is clearly contextual, depending on both the cell type along with the physiological or patho-physiological status of the tissue microenvironment. On epithelial cells and several leukemia/lymphoma cells, activation of Mertk, like many RTKs, triggers canonical RTK post-receptor signaling that includes stimulation of ERK1/2, Akt, YAP/TAZ, and p38 MAP kinases that contribute to cell invasion, migration, angiogenesis, cell survival, chemoresistance, and metastasis, as noted in excellent reviews from Cummings et al., Huelse et al., and others (Cummings et al., 2013; Huelse et al., 2020). This paradigm is nicely illustrated with respect to NF-κB, whereby in tumor cells, Mertk activates NF-κB to enhance survival signaling whereas in myeloid immune cells (including macrophages), Mertk inhibits NF-κB and dampens the pro-inflammatory cytokine response (Camenisch et al., 1999; Lew et al., 2014; Pagani et al., 2020). In addition to canonical RTK signaling pathways, Mertk can activate non-canonical signals on epithelial cells that impinge on tumor immunity. For example, we have previously shown that activation of Mertk can result in Akt-dependent upregulation of PD-L1 on tumor cells (Kasikara et al., 2017). Finally, it is interesting to note that Mertk may cooperate with other members of the TAM family. A good example of this was recently shown by Davra et al. where it was found that an Axl-driven cancer cell line (E0771) and a Mertk global knockout murine model independently reduced tumor burden, increased overall survival, and led to tumor-specific T cell responses. From within the same study, when Mertk knockout mice were combined with Axl knockout E0771 cells, the individual benefits were additive (Davra et al., 2020). These data also

indicate that TAMs on tumor cells and tumor-associated macrophages can have distinct, non-overlapping functions to alter tumor progression.

2.2 Efferocytosis

On both professional phagocytes, such as macrophages, and non-professional phagocytes, Mertk is one of the pre-eminent receptors responsible for mediating binding and phagocytosis of apoptotic cells, a process known as efferocytosis (Scott et al., 2001). At the biochemical and cell biological level, Mertk-mediated efferocytosis activates tyrosine phosphorylation-dependent reorganization of the actin cytoskeleton by both Rac1 and Vav1 guanine nucleotide exchange pathways (Mahajan and Earp, 2003). In the Vav1-dependent pathway, studies have shown that tyrosine phosphorylated Vav1 activates Rho family members Rac1, CDC-42, and RhoA that spatially regulate the actin cytoskeleton to promote efferocytosis (Crespo et al., 1997; Mahajan and Earp, 2003) (see excellent review Myers et al., 2019). In the Rac1 pathway, stimulation of Mertk induces Src-mediated crosstalk to αVβ5 integrin (a ligand for the PS bridging molecule MFG-E8) to promote actin reorganization (Wu et al., 2005).

2.3 Cooperation with TIM-4 for PS recognition

Such cooperation of PS receptors is also evident by studies showing that Mertk can cooperate with TIM-4, a member of T cell transmembrane, immunoglobulin, and mucin family of receptors (TIM-1, TIM-3, and TIM-4) to induce efferocytosis of resident macrophages (Nishi et al., 2014, 2019). These studies showed that Mertk-mediated efferocytosis may be aided by “tethering” receptors (TIM-4) that initiates PS-specific binding to an apoptotic cell but also lacks a signaling domain. Meanwhile, Mertk is capable of initiating post-receptor signaling and engulfment, illustrating a concept first proposed by Somersan et al. (Ravichandran, 2011; Somersan and Bhardwaj, 2001) (Fig. 2). While these data are consistent with the idea of cooperation between different PS receptors to drive apoptotic cell recognition and efferocytosis, mutagenesis studies substituting Tyr → Phe in the autophosphorylation docking sites have shown that Mertk-driven rearrangement of the actin cytoskeleton can be dissociated from the Mertk-mediated suppression of NF-κB via post-receptor intracellular signaling (Tibrewal et al., 2008).

3. Immunology of Mertk activation

With respect to the immunological roles of Mertk, it is well established that events associated with efferocytosis are tolerogenic, characterized by the enhanced production of immunosuppressive cytokines (IL-10, TGF-β, and IL-4) associated with M2 macrophages (Birge et al., 2016; Huynh et al., 2002; Savill and Fadok, 2000; Savill et al., 2002; Vaught et al., 2015). Concomitantly, classical efferocytosis by macrophages also results in the decreased production of immune-stimulating cytokines associated with M1-like macrophages such as IL-12, TNF-α, and IL-1β (Deng et al., 2012; Kim et al., 2004; Sen et al., 2007). Finally, efferocytosis and the degradation of the ingested cellular lipids, engage nuclear receptors, such as retinoid X receptors (RXRs) and peroxisome proliferator activated receptors (PPARs), and further drive M2 macrophage polarization markers as well as the upregulation of Mertk in a feed-forward loop (Rőszer et al., 2011; Zizzo and Cohen, 2015).

In addition to promoting efferocytosis, activation of Mertk triggers two integrated but biologically distinct responses, anti-inflammation and pro-resolution. The anti-inflammatory response, while not completely understood, is mediated by a post-receptor pathway that leads to the inhibition of NF-κB-mediated signaling and nuclear translocation (Cvetanovic and Ucker, 2004). In an independent Mertk post-receptor pathway in macrophages, Mertk signaling promotes SPM biosynthesis and enhances resolution by decreasing the levels of 5-LOX phosphorylated at Ser271 (p-Ser271–5-LOX) and increasing levels of cytoplasmic 5-LOX (Cai et al., 2020). Together, Mertk expression on both epithelial cells and myeloid cells (particularly macrophages) drives a series of post-receptor signals that impinge on tumorigenesis and immune evasion. Furthermore, as noted above, because Mertk signaling is amplified in the presence of externalized PS, events associated with Mertk activation, including tolerance, suppression of inflammation, and inflammation resolution, are expected to become exaggerated in the stressed TME where constitutively expressed PS is typically observed, such as in solid tumors (Fig. 3). Constitutively high PS within solid tumors occurs due to the high apoptotic index of proliferating cells and aberrant activation of PS scramblases, such as TMEM16F, that continually scramble and expose PS in the TME (Birge et al., 2016; de Jong et al., 2000). As further emphasized below, this rationale that Mertk is preferentially activated in the TME, predicts that Mertk may be a universal tumor antigen or biomarker for the development of targeted therapies.

4. Rationale for targeting Mertk as a therapeutic

4.1 Role of Mertk in immune modulation of the TME

The immunological state of the TME can be a major determinant in outcomes of immunotherapy clinically. Factors such as immune cell infiltration, cytokine expression, macrophage polarization, and T cell activation greatly affect the response to immunotherapy (Bai et al., 2020). “Hot” tumors, tumors that have a pro-inflammatory state and are infiltrated by T cells, are more likely to respond to immunotherapy than “cold” tumors, tumors which have an immune-suppressive state and reduced T cell infiltration (Liu and Sun, 2021). Efferocytosis itself and efferocytosis-mediated tolerance can polarize the TME to a “cold” phenotype, thus promoting tumor progression and immune checkpoint blockade resistance (Kumar et al., 2017). As Mertk is a major efferocytic receptor, overexpression may induce efferocytosis on tumor cells that would not normally be efferocytic, whereas appropriately polarized immunosuppressive macrophages expressing Mertk are also highly efferocytic (Nguyen et al., 2014; Seitz et al., 2007). In the context of cancer, reducing efferocytosis, and thus targeting Mertk, promotes a TME that permits increased immune activity and T cell infiltration (Davra et al., 2020; Lindsay et al., 2021; Zhou et al., 2020). Thus, Mertk activation can be seen as a brake on pro-inflammatory cytokines whereas Mertk blockade is akin to releasing the brake and activating the pro-inflammatory cytokines.

4.2 Mertk knockout models

The physiological role of Mertk is apoptotic cell recognition, and its activation by PS leads to efferocytosis and resolution of inflammation. Historically, the capacity for Mertk to act as a homeostatic receptor and dampen inflammatory responses in tissues was first demonstrated by the phenotype of Mer kinase-dead (MerKD) mice, whereupon the

administration of a sub-lethal LPS dose was characterized by excessive TNFα production and death by endotoxic shock (Camenisch et al., 1999). Mertk knockout mice also showed altered macrophage cytokine production and developed a progressive lupus-like autoimmunity (Cohen et al., 2002). From these initial studies, Mertk, having been characterized as a dominant efferocytosis receptor and possessing strong inflammation resolution functions, was rightly predicted to play a major role in immune suppression and tumor progression in tumors. Translating to cancer biology, in an interesting study by Cook et al., breast cancer, melanoma, and colon cancer tumors progressed slowly and metastasized lesser in Mertk$^{-/-}$ mice. Within the same study, lethally irradiated mice with transplanted Mertk$^{-/-}$ bone marrow experienced altered cytokine production by CD11b^{+} leukocytes that ultimately reduced tumor growth. Mertk$^{-/-}$ leukocytes expressed lower pro-resolution cytokines and signals such as IL-10 and Gas6 and higher pro-inflammatory cytokines such as IL-12, and IL-6, effectively skewing the TME toward pro-inflammatory phenotype. This effect was accompanied by CD8^{+} T cell infiltration and proliferation (Cook et al., 2013). Considering these observations, Mertk blockade therapies were predicted to activate adaptive immunity by promoting production of inflammatory cytokines involved in T cell stimulation.

4.3 Role of Mertk in macrophage polarization

M2c macrophages, polarized by IL-10, primarily have a wound healing and immunosuppressive phenotype and secrete associated cytokines including IL-10 and TGF-β, as well as express Mertk (Zizzo et al., 2012). Therefore, the presence of tumor-associated Mertk^{+} M2c macrophages is undesirable as these cells contribute to immune suppression and tumor growth (Chen et al., 2021). Importantly, Mertk activation also drives M2c polarization itself, further supporting tumor growth (Martinez et al., 2006; Zizzo et al., 2012). Blocking Mertk by antibody or small molecules has been shown to reduce M2c polarization (Rios-Doria et al., 2020). Less established, though equally as important, is the aspect of tumor antigen presentation within the TME. While macrophages do possess an ability to present antigens, MHC expression is typically restricted to M1 inflammatory macrophages and not the M2c Mertk^{+} macrophage (Tariq et al., 2017). Therefore, Mertk-mediated efferocytosis may lead to the uptake of apoptotic cellular debris that may otherwise be presented as tumor antigens by dendritic cells or other antigen-presenting cells. Consequently, tumor antigens are not cross-presented to CD8^{+} T cells, dampening the adaptive T cell responses. Thus, in addition to obvious therapeutic benefits from reducing Mertk-mediated signaling, as suggested from earlier studies, therapeutic targeting of Mertk may lead to an enhanced tumor-specific adaptive immune response that would ultimately decrease tumor burden and the incidence of relapse via an abscopal effect (Davra et al., 2020; Zhou et al., 2020).

4.4 PD-L1 regulation by Mertk

Mertk also regulates an important immune checkpoint, the PD-1/PD-L1 axis. PD-1 is commonly expressed on T cells and interacts with the PD-L1 receptor on macrophages to inhibit T cell activation. Nguyen et al. showed that constitutively active Mertk increased PD-L1 and PD-L2 expression while Kasikara et al. confirmed this finding by showing that PS-sensing by Mertk was necessary to induce PD-L1 expression in breast cancer cells

(Kasikara et al., 2017; Nguyen et al., 2014). Several studies have later shown that Mertk blockade enhances anti-tumor effects of PD-L1 therapy, and these pathways work in synergy with each other (Davra et al., 2020; Du et al., 2021; Zhou et al., 2020).

5. Therapeutic strategies and current progress

5.1 Kinase domain-targeting using small molecule inhibitors

For the reasons mentioned above, there is continued interest to develop next generations of Mertk tyrosine kinase inhibitors (TKIs) with improved specificity, most notably for applications in leukemias, where Mertk is clinically associated with poorer prognosis and shorter overall survival and is ectopically expressed in up to 50% of acute lymphoblastic leukemias (ALL) and over 80% of acute myeloid leukemias (AML) (Huey et al., 2016; Keating et al., 2006; Lee-Sherick et al., 2018; Linger et al., 2013). Presently, there are several candidate TKIs for Mertk in clinical trials that provide specificity (to varying degrees), membrane permeability, and accessibility to the intracellular kinase domain (Table 1). TKIs, by binding to the ATP-binding pocket in the kinase domain, block the downstream signaling cascade and directly inhibit the autophosphorylation process proceeding dimerization (Fig. 4, top left). For example, UNC2025, an orally-bioavailable small molecule with suitability for clinical application, induces significant disease progression in bone marrow and patient-derived AML xenograft models (Cummings et al., 2015). Though currently all Mertk therapies in clinical trials consist of small molecule TKIs, challenges have arisen from anticipated as well as unexpected off-target effects due to the highly conserved kinase domain of tyrosine kinase receptors. As noted in Table 1, currently all of the clinically approved Mertk therapies have secondary targets. However, their excellent bioavailability and in vivo efficacy offer important advantages lacking with other therapeutic methods. The development of more highly selective Mertk inhibitors would be a welcomed advance to limit off-target effects, particularly as the idea to target macrophages in cancer immuno-oncology applications continues to gain traction.

5.2 Mertk degraders

As an extension of small molecule TKIs that target Mertk, degraders offer an alternative approach to target the Mertk kinase domain and limit the presence of the intact receptor on the surface, in contrast with classic inhibitory models that would otherwise leave receptors intact. Rather than interfering with ligand binding, dimerization, autophosphorylation, or activation, degrader molecules, also known as PROTAC (**PRO**teolysis **TA**rgeting **C**himeric) molecules, inhibit receptor activity by linking a targeting domain to an E3 ligase that ubiquitinates and marks the target protein for proteasomal degradation (Fig. 4, top, second from left). Targeting Mertk with degraders could have promising therapeutic potential as PROTAC drugs are now beginning to enter clinical utility. PROTAC inhibitors can also bypass compensatory stabilization and upregulation of receptor levels that can occur with other small molecule inhibitors. As mentioned above, with the development of more specific Mertk inhibitors, the concept and practical use of Mertk degraders will be better realized. As a proof of concept for TAM receptors, degraders developed by Kim et al. that target Axl have shown to delay and overcome resistance in NSCLC in humans in combination with EGFR TKIs, suggesting promise to this experimental approach (Kim et al., 2019).

5.3 Mertk blocking antibodies

In addition to small molecule TKIs and degraders, in recent years several groups have demonstrated preclinical utility of anti-Mertk antagonistic monoclonal antibodies (mAbs). While antagonistic mAbs have not yet been reported in human clinical trials, such mAbs might be expected to have greater specificity than TKIs (Fig. 4, top, second from right). Using an immune-competent murine model of breast cancer (E0771), Davra et.al showed reduced tumor growth and metastasis when mice were administered an anti-Mertk neutralizing mAb. Furthermore, the anti-Mertk mAb acted synergistically with anti-PD1 checkpoint therapeutics and phenocopied effects of Mertk ablation, suggesting that Mertk can be pharmacologically targeted with mAbs. Mechanistically, knockout of Mertk or mAb blockade of Mertk resulted in decreased macrophage efferocytosis, decreased M2 macrophages, and increased $CD8^+$ T cell infiltration into the tumor, suggesting that Mertk-blocking antibodies can stimulate host anti-tumor immunity (Davra et al., 2020). Similar studies were recently reported by Zhou et al. using MC38, a mouse model of colon cancer and B16 melanoma models whereby Mertk blockage acted synergistically with anti-PD1 therapeutics. These authors also showed that Mertk mAbs block macrophage efferocytosis, and in doing so, uncleared and accumulated dying cells undergo secondary necrosis and subsequently release DNA that activates the STING and IFN pathways in tumor-associated macrophages (Zhou et al., 2020). Finally, Ceatano et al. investigated the effects of triple therapy by combining Mertk blockade, PD-1 inhibition, and radiation therapy in a murine metastatic adenocarcinoma NSCLC model (Caetano et al., 2019). Importantly, these studies showed not only that triple therapy reduced tumor progression, but it also activated $CD8^+$ T cells, NK cells, and tissue-resident memory cells. Taken together, these preclinical studies in immunocompetent murine models support the rationale that pharmacological Mertk blockade with mAbs, either in combination with checkpoint inhibitors or radiation therapy, shifts the immunological landscape of the TME from immunosuppressive to immunogenic and activates the adaptive immune responses necessary for long term anti-tumor immunity. While possible caveats of anti-Mertk neutralizing mAbs might include retinal toxicity due to impaired Mertk function in the retinal pigmented epithelium, these effects could be partially mitigated by both the acute utilization of mAbs or by the design of bi-specific Mertk antibodies or pH sensitive antibodies that preferentially bind targets at lower pHs in the TME. Finally, while the above arguments focus on antagonistic mAbs that block macrophage efferocytosis and enhance immunogenic outcomes, targeted Mertk by agonistic mAbs might conversely have beneficial effects in autoimmunity, such as lupus, where activated monocytes contribute to inflammation and hyperactivation.

5.4 Wedge inhibitors

An independent small molecule strategy to target Mertk and TAMs has been proposed based on key structural features of TAM ligand-interacting ectodomains. From studies investigating the crystal structure of Axl, it is known that Gas6 and ProS1 interact with the tandem Ig-1 (major contact) and Ig-2 (minor contact) domains to trigger receptor dimerization and activation (Meyer et al., 2015; Sasaki et al., 2006). Based on iterative computer simulations, small molecule wedge inhibitors were screened and predicted to target the extracellular domain of Axl at the interface of the Ig-1 ectodomain of Axl and the LG-1 of Gas6. While still early in development, these molecules (RU301 and RU302)

are predicted to “wedge” between ligand and receptor and prevent a conformational change required for receptor activation (Kimani et al., 2017) (Fig. 4, top right). Advantages of these molecules include targeting and utilization of an extracellular epitope that does not require membrane permeability as well as expected reduced off-target effects of TKIs since they target a more unique Ig1 domain as opposed to the kinase domain used by other inhibitors.

5.5 Soluble traps

Previous studies have shown that full-length Mertk is downregulated by an ADAM17-mediated ectodomain cleavage event producing a soluble fragment (soluble Mertk; sMertk) that comprises the ligand-binding extracellular domain (Thorp et al., 2011). The physiological relevance of sMertk is supported by evidence stemming from in vivo studies that show significant levels of serum sMertk and in vitro studies that showed the addition of exogenous sMertk dose-dependently inhibits Mertk-mediated phagocytosis (Sather et al., 2007; Wu et al., 2011). Such studies suggest sMertk is not just a cleavage byproduct but is physiologically important in regulating Mertk activity, likely by acting as a decoy receptor to sequester free ligand, thereby competing with membrane-bound receptors to tonically reduce Mertk activity (Fig. 4, bottom left). Strategies aimed to inhibit intact, membrane-bound Mertk could involve either recombinant biologicals containing the ectodomain of Mertk or other molecular structures designed to sequester Gas6. A similar strategy has been reported by Kariolis and colleagues in which the authors created a recombinant high-affinity Fc-Axl ectodomain with femtomolar affinity for Gas6. The molecule effectively acted as a “decoy receptor” and was reported to lower serum Gas6 levels and reduce metastasis in an ovarian tumor model (Kariolis et al., 2014). Utilizing the structure of the Axl Ig domains may be more appropriate for soluble trap therapeutics as Axl only has affinity for Gas6 and not Protein S (Tsou et al., 2014). Sequestration of the latter should be avoided due to its role in the coagulation cascade. Still, it should be noted that Gas6 sequestration would effectively serve as a panTAM inhibitor and not specific for Mertk.

5.6 Mertk inhibition via inhibiting PS binding

Another potentially interesting strategy to block Mertk signaling could be generated from findings that both active Gas6 and ProS1 have an absolute requirement for Vitamin K-dependent γ-carboxylation of the Gla domain to permit PS binding (Fig. 4, bottom, second from right; bottom right). Indeed, our observations that γ-carboxylation is required for activity but not receptor binding posits that such recombinant biologicals produced in presence of Warfarin are expected to compete with γ-carboxylated/active ligands and prevent ligand-PS binding and Mertk dimerization/activation (Tsou et al., 2014). The molecular mechanism or structural explanation as to why non-γ-carboxylated ligand fails to activate Mertk remains to be elucidated, including why Mertk ligands require PS for activity amplification. Regardless, non-γ-carboxylated/inactive ligand may also have an unanticipated activity to block Mertk signaling and PS-mediated enhancement in the TME. In addition to the potential therapeutic utility of non-γ-carboxylated ligand, low-level Warfarin administration itself, used commonly as an anticoagulant therapy, may have an unanticipated consequence to lower Gas6 and ProS1 γ-carboxylation, and thus Mertk activity, globally. Interestingly, warfarin treatments have been anecdotally associated with decreasing cancer risk, supporting the premise that Mertk inactivation by non-γ-

carboxylated ligands would impede potential tumor growth. (Haaland et al., 2017; Kirane et al., 2015).

6. Concluding remarks and future perspectives

Historically, the importance for Mertk to act as a homeostatic receptor and dampen inflammatory responses in tissues was first demonstrated by the phenotype of Mer kinase-dead (MerKD) mice, characterized by an excessive production of TNFα upon LPS stimulation and death by endotoxic shock caused by sublethal doses of LPS (Scott et al., 2001). More recently, preclinical studies focused on immuno-oncology applications have associated Mertk with immune evasion and tumor progression. Inhibition of Mertk in several tumor models is associated with reduced immunosuppressive M2 macrophages, increased T cell infiltration into tumors, and synergy with anti-PD-1 checkpoint therapeutics (Crittenden et al., 2016; Davra et al., 2020; Zhou et al., 2020). However, important information about how Mertk acts as a myeloid inhibitory protein is still missing. Further exploring the physiological roles of Mertk and underlying molecular mechanisms will provide novel insights regarding how it functions pathologically under conditions of highly dysregulated PS in the TME. Additional research on Mertk biology, namely, its proteolytic processing, regulation of expression in macrophages, role in macrophage polarization, and how it impinges on PD-L1 levels, may contribute towards predicting early pathological conditions and immune escape potential. Clearly, exploiting Mertk is a very promising avenue of research that could lead to novel therapeutic targets, where the expression and/or activity could contribute to the treatment of solid cancer.

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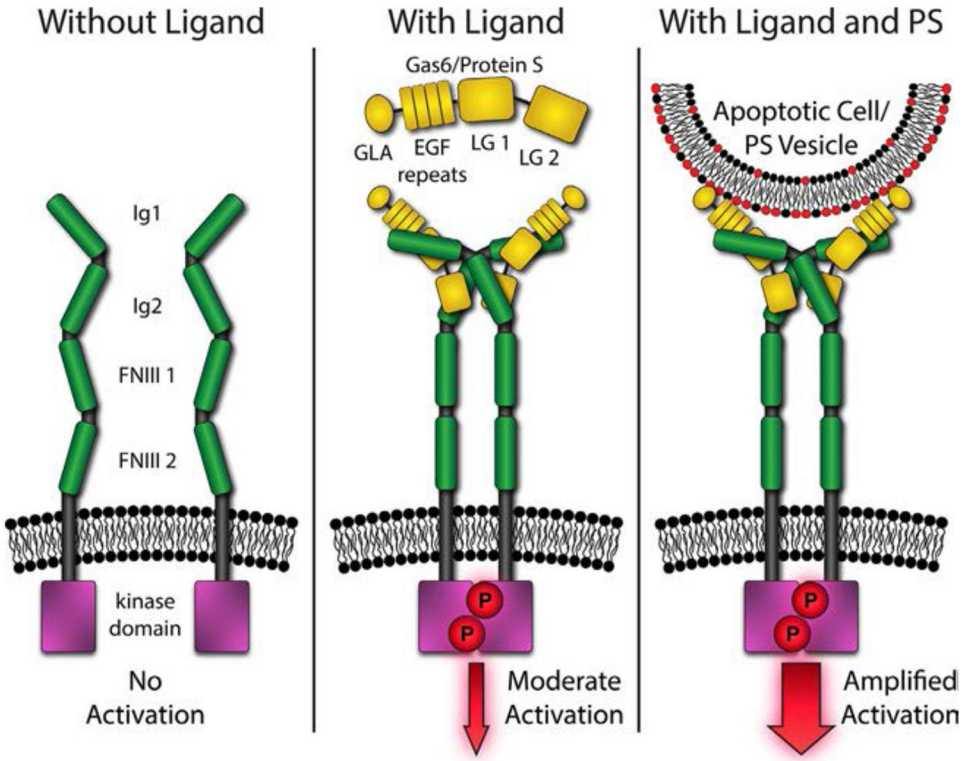

Fig. 1.

Mertk activation by ligands and PS. Mertk consists of two immunoglobulin-like C2-type (Ig) domains, two fibronectin-type III (FNIII) domains, followed by a transmembrane domain and an intracellular kinase domain (left). LG domains of Gas6 and Protein S bind to the Mertk Ig domains and leads to dimerization, autophosphorylation, and moderate activation (middle). Signaling is amplified with the addition of PS, which binds to the γ-carboxylated Gla domain on Gas6 and Protein S (right).

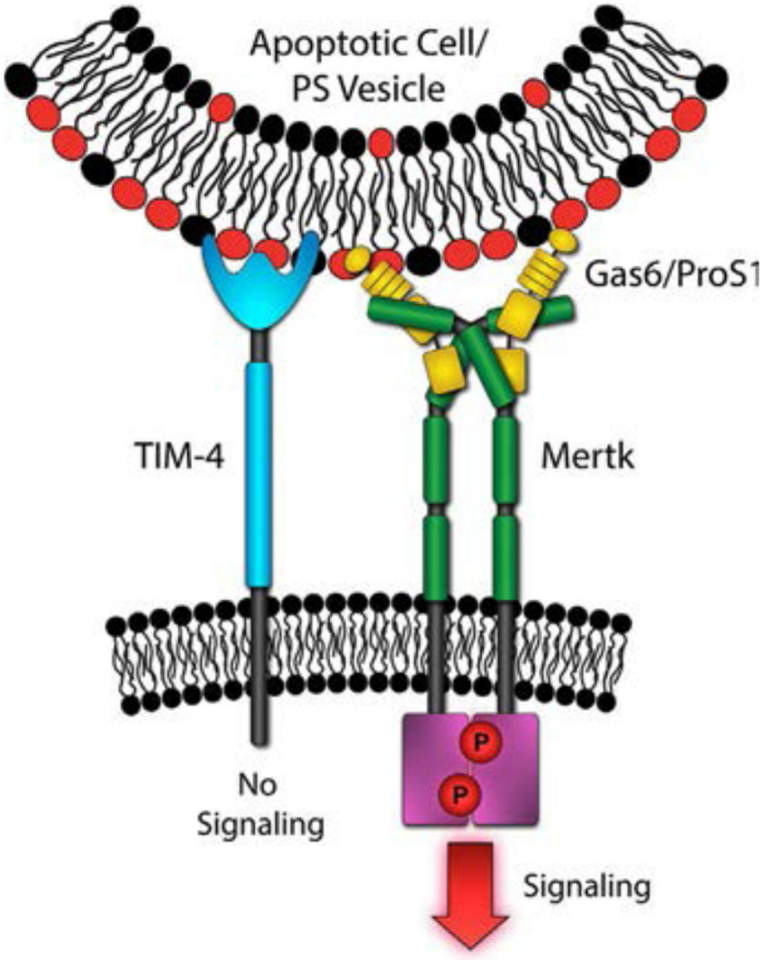

Fig. 2.

Tethering and tickling receptors, TIMs and TAMs. TIM receptors, such as TIM-4, are considered “tethering” receptors and directly bind to PS via the IgV domain while TAM receptors, such as Mertk, are considered “tickling” receptors and signal through ligand-mediated PS-binding. Despite the different mechanisms of PS-binding, TIM-4 and Mertk cooperate to enhance efferocytosis.

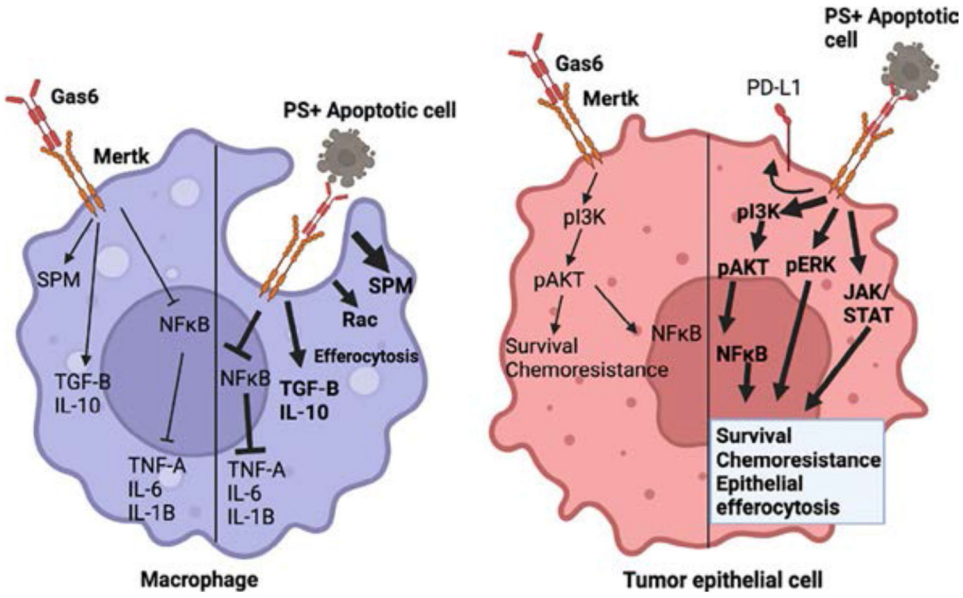

Fig. 3.

Mertk activation is amplified by PS. In the absence of PS, Gas6 binding of Mertk moderately activates the receptor. Left: Upon recognition of PS externalized on apoptotic cells, macrophages expressing Mertk become hyperactivated and exert stronger immune suppressive effects by Rac-mediated efferocytosis, TGF-β, IL-10, and SPM secretion and pro-inflammatory cytokine (TNF-α, IL-1β and IL-6) suppression. Right: In Mertk-expressing epithelial cells, PS recognition leads to pAKT-mediated chemoresistance, survival, and PD-L1 expression.

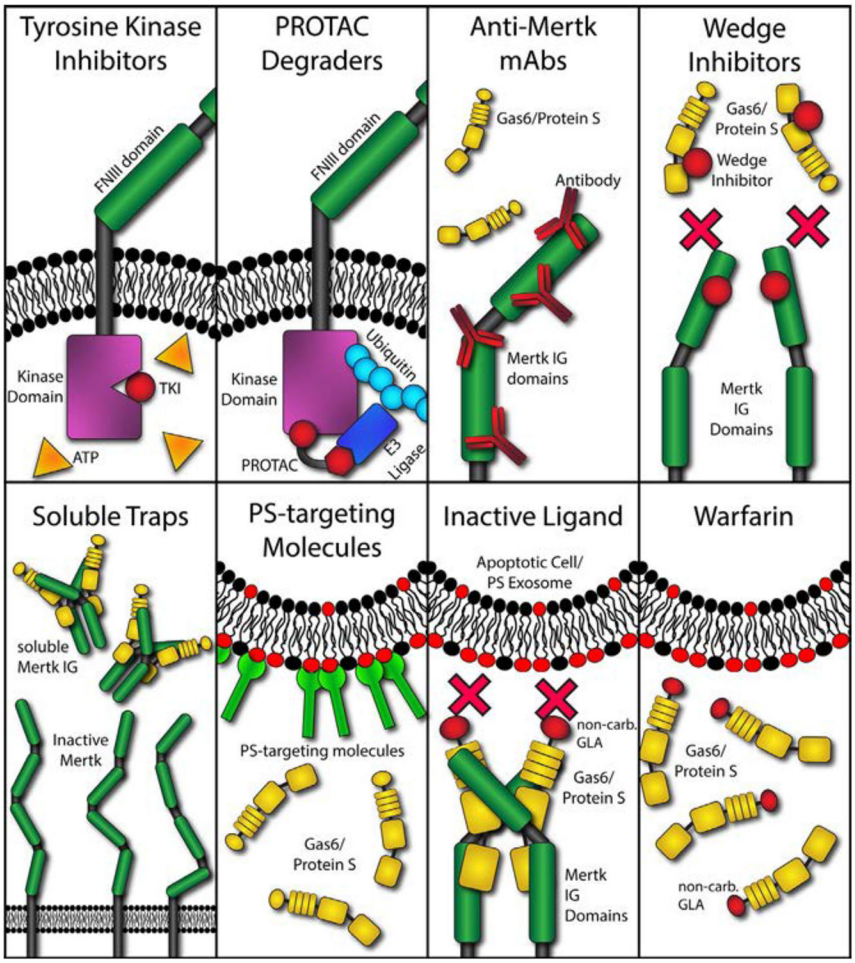

Fig. 4.

Strategies for anti-Mertk therapeutics. A variety of strategies are possible for targeting Mertk therapeutically. Small molecule inhibitors bind to tyrosine kinase domain in competition with ATP to prevent Mertk autophosphorylation after dimerization/activation. As of this writing, small molecule tyrosine kinase inhibitors are the only class currently in clinical trials. PROTAC degraders target the intracellular domain for ubiquitin-mediated proteolysis, and uniquely inhibit signaling by reducing the amount of intact receptors. Anti-Mertk antibodies inhibit Mertk activation by binding to the Ig domains of Mertk and preventing ligand binding or the resulting conformation changes that result in autophosphorylation. Wedge inhibitors target the Gas6/Mertk interface at the LG/Ig domains to prevent activation. Soluble traps consist of soluble Mertk ectodomain or other molecular iterations that act as decoy receptors to compete with membrane-bound, intact Mertk for soluble Gas6 molecules. PS-targeting molecules opsonize PS and prevent PS-mediated activation. Inactive ligand lacks γ-carboxylation of the Gla domain and is unable to bind PS and activate Mertk. The utilization of Warfarin globally prevents γ-carboxylation of the Gla domain, PS-ligand binding, and Mertk activation.

Table 1

Mertk therapeutics in the clinic.

Mertk therapeutic (Alternative targets)	Combination treatment	Clinical trials	Phase	Disease targets
MRX-2843 (FIt3)	Osimertinib	NCT04762199	Phase 1b	Advanced Lung Non-Small Cell Carcinoma
				Metastatic Lung Non-Small Cell Carcinoma
				Stage IV Lung Cancer AJCC v8
				Stage IVA Lung Cancer AJCC v8
				Stage IVB Lung Cancer AJCC v8
	N/A	NCT04946890	Phase 1/2	Acute Myeloid Leukemia
	N/A	NCT03510104	Phase 1	Advanced Cancer
				Metastatic Cancer
				Neoplasms
				Neoplasm Metastasis
				Neoplastic Processes
				Pathologic Processes
	N/A	NCT04872478	Phase 1	Acute Myeloid Leukemia
				Acute Lymphoblastic Leukemia
				Mixed Phenotype Acute Leukemia
ONO-7475 (Axl)	Venetoclax (Bcl-2 blocker)	NCT03176277	Phase 1/2	Acute Leukemia
				Myelodysplastic Syndromes
	Nivolumab (ONO-4538; PD-1 inhibitor)	NCT03730337	Phase 1	Advanced or Metastatic Solid Tumors
PF-07265807 (Axl)	Sasanlimab (PD-1 inhibitor) Axitinib (VEGFR, c-KIT, PDGFR inhibitor)	NCT04458259	Phase 1	Neoplasm Metastasis
Q702 (Axl; CSF1R)	N/A	NCT04648254	Phase 1	Solid Tumor
				Advanced Cancer
				Metastatic Cancer
RXDX-106 (Tyro3, Axl cMET)	N/A	NCT03454243	Phase 1 (Terminated)	Advanced or Metastatic Solid Tumors
INCB081776 (Axl)	Retafanlimab (INCMGA00012; PD-1 inhibitor)	NCT03522142	Phase 1	Advanced Solid Tumors

A range of small molecule tyrosine kinase inhibitors are under evaluation in Phase 1 and Phase 2 clinical trials targeting a variety of cancers.