MER, Melanoma and Inhibitors

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This is an examination of a specific Tyrosine Kinase receptor and its inhibition as a means for Melanoma metastatic control. Copyright 2013 Terrence P. McGarty, all rights reserved.

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1 INTRODUCTION

The focus on pathways, receptors, ligands and promoters as control elements for cancer has seen a great deal of development in the past decade. One key approach is the development and identification of inhibitors, molecules which can block an over excited pathway. We examine here a specific recent such example as relates to melanoma. It is already well known that BRAF suppression is an effective approach albeit often of limited duration. The development of inhibitors for a selection of evolving pathway aberrations will most likely be the way to turn a deadly disease into a chronic but manageable problem, assuming that one can get permission to use such molecules, a process which not is costly and lengthy.

We use a recent paper by Schlegel et al and use MER as a prototypical example of pathway control via inhibitor blockage.

MER is a tyrosine kinase ("TK") receptor ("TKR")[1]. As Marks et al state there are 85 members in the TK family and 58 of these are receptors. The receptors are divided into various families based upon their structures and one family contains Axl, Sky and MER, also known as the TAM family[2]. This family, as we shall see, has immunoglobulin like regions on the outside of the cell surface and kinase domains on the inner surface. The family also has a dual fibronectin III-like domain on the outside just below the immunoglobulin domains, of which there are two.

Generally the receptors are activated by ligands which in turn result in the phosphorlyation of the kinase region and associated area and then commence the activation of the related pathways. Now these pathways are the ones that result in proliferation and loss of localization and thus result ultimately in metastasis.

We use this example for two reasons: (i) it is a good example to demonstrate the activation of pathways and metastatic growth; (ii) it also is a good example of how inhibitors can function on receptors and thus can inhibit metastatic growth.

As Schlegel et al state:

Receptor tyrosine kinases (RTKs) are frequently ectopically expressed, overexpressed, or hyperactivated in tumor cells and are therefore attractive targets for cancer therapy. C-MER proto-oncogene tyrosine kinase (MERTK), a member of the TAM (TYRO, AXL, MERTK) family of RTKs, has been characterized as a therapeutic target in hematopoietic malignancies and several solid tumors including lung, prostate, and brain

There is a subtle question posed but not answered here. Is it over-expression, and if so by what ligand, or is it an excess production of MER and thus an over-expression. What is the status of the benign cell, and is this the dominant pathway. Clearly by having too active or too many MER

[1] From NCBI we have (2q14.1): *This gene is a member of the MER/AXL/TYRO3 receptor kinase family and encodes a transmembrane protein with two fibronectin type-III domains, two Ig-like C2-type (immunoglobulin-like) domains, and one tyrosine kinase domain*.

[2] TAM, (**T**YRO, **A**XL, **M**ERTK)

receptors, actually any TAM like receptor will do, leads to proliferation. This goal of blocking the receptor so that it does not start the process is a valid approach.

The authors clearly state:

Stimulation of melanoma cells with the MERTK ligand GAS6 resulted in the activation of several downstream signaling pathways including MAPK/ERK, PI3K/AKT, and JAK/STAT. MERTK inhibition via shRNA reduced MERTK-mediated downstream signaling, reduced colony formation by up to 59%, and diminished tumor volume by 60% in a human melanoma murine xenograft model.

Namely we have a ligand, GAS6, which activates the MER pathway. Is that ligand over expressed. On the other hand the molecule shRNA reduced the activation.

They specifically state:

In addition, Sensi et al. found that melanoma cells often secrete GAS6, a ligand of TAM receptors, indicating a mechanism of TAM autocrine signaling in melanoma…. The mechanism of MERTK activation in melanoma cells is not clear, but Sensi et al. have previously described melanoma cell expression and secretion of GAS6, the common ligand for all members of the TAM family of proteins, suggesting a method of autocrine and/or paracrine activation of MERTK. Since expression of MERTK by melanoma cells increases during progression from primary to metastatic melanoma, it would be interesting to determine whether corresponding increases in GAS6 levels occur in serum from patients with metastatic melanoma, implicating serum GAS6 levels as a potential early marker of melanoma progression, as in other cancers.

Thus possibly inhibiting GAS6 may be profitable as well[3]. However the focus here is receptor inhibition.

1 MER AND MELANOMA

Let us consider a recent development in understanding MER and melanoma. We return to the recent paper by Schlegel et al where the author's state:

C-MER proto-oncogene tyrosine kinase (MERTK) is a receptor tyrosine kinase with oncogenic properties that is often overexpressed or activated in various malignancies. Using both protein immunohistochemistry and microarray analyses, we demonstrate that MERTK expression correlates with disease progression.

MERTK expression was highest in metastatic melanomas, followed by primary melanomas, while the lowest expression was observed in nevi. Additionally, over half of melanoma cell lines

[3] As NCBI states: *This gene product is a gamma-carboxyglutamic acid (Gla)-containing protein thought to be involved in the stimulation of cell proliferation, and may play a role in thrombosis. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.* Located at 13q34. http://www.ncbi.nlm.nih.gov/gene/2621

overexpressed MERTK compared with normal human melanocytes; however, overexpression did not correlate with mutations in BRAF or RAS.

Stimulation of melanoma cells with the MERTK ligand GAS6 resulted in the activation of several downstream signaling pathways including MAPK/ERK, PI3K/AKT, and JAK/STAT. MERTK inhibition via shRNA reduced MERTK-mediated downstream signaling, reduced colony formation by up to 59%, and diminished tumor volume by 60% in a human melanoma murine xenograft model.

Treatment of melanoma cells with UNC1062, a novel MERTK-selective small-molecule tyrosine kinase inhibitor, reduced activation of MERTK-mediated downstream signaling, induced apoptosis in culture, reduced colony formation in soft agar, and inhibited invasion of melanoma cells. This work establishes MERTK as a therapeutic target in melanoma and provides a rationale for the continued development of MERTK-targeted therapies.

Thus, like to work that led to BRAF V600 inhibitors, we see MER TK is another interesting target. The authors also provide an inhibitor molecule as well.

2 TYROSINE KINASES AND MER

Tyrosine Kinases receptors have received a great deal of attention especially in the area of cancer metastasis and in cancer control. They are as Verma et al state:

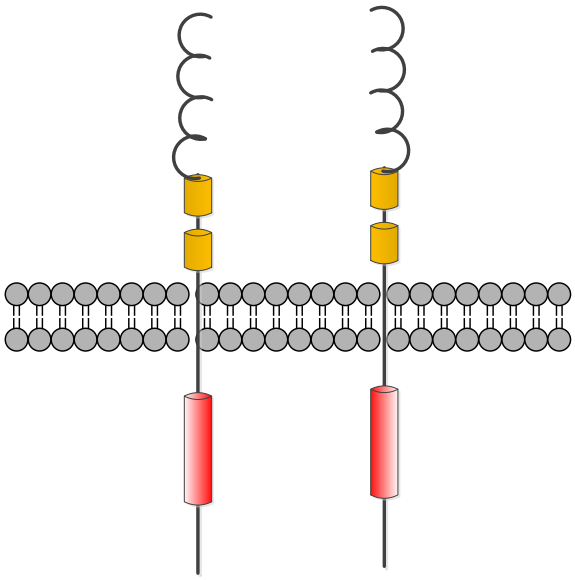
Receptor tyrosine kinases (RTK) are a large family of transmembrane proteins exhibiting great diversity in their extracellular regions, although sharing in common a highly conserved intracellular tyrosine kinase domain. They function as sensors for extracellular ligands, the binding of which triggers receptor dimerization and activation of the receptor's kinase activity. This activation leads to the recruitment, phosphorylation, and activation of multiple downstream signaling proteins, which ultimately change the physiology of the cell. RTKs regulate cellular processes, including survival, growth, differentiation, adhesion, proliferation, and motility. Fifty-eight known RTKs in the human genome are classified into 20 families by amino acid sequence identity within the kinase domain and structural similarities within their extracellular regions.

There are many such tyrosine kinase receptors. One class is the TAM family and as Verma et al state:

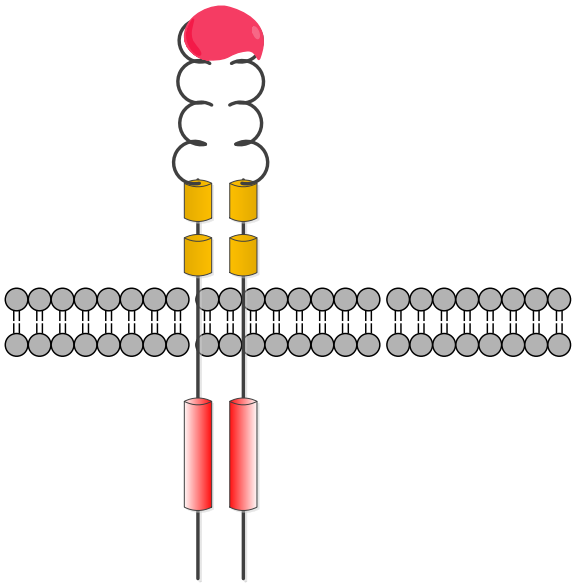
One subfamily is referred to as the TAM family, identified in 1991, comprising Tyro-3 (also called Sky), Axl, and Mer. The TAM receptors are characterized by a combination of 2 immunoglobin-like domains and dual fibronectin type III repeats in the extracellular region and a cytoplasmic kinase domain. The primary ligand for TAM receptors is growth arrest-specific 6 (Gas 6), a fairly large (75 kDa) vitamin K–dependent protein known to activate downstream signaling

We depict a simple structure below containing the elements specified above.

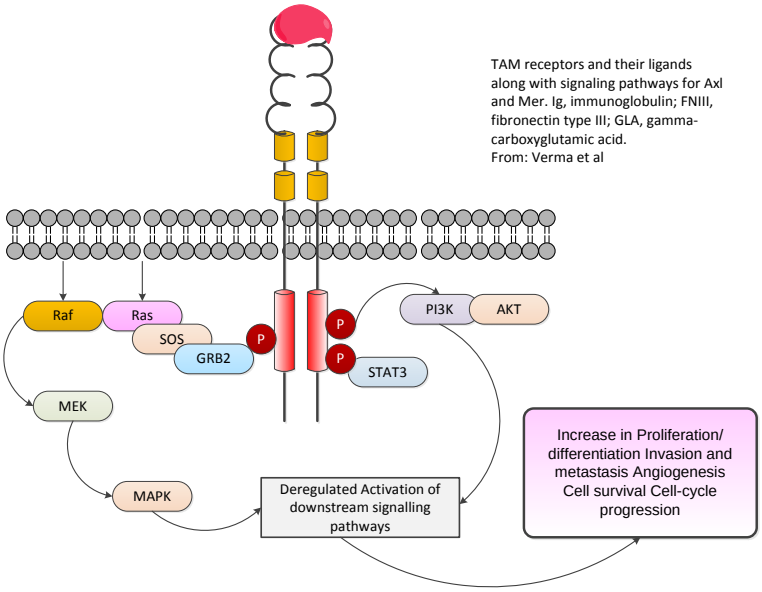
Let us consider a simple development of MER controlled pathways. The Figure below shows two separate and un-activated MERTK molecules with the immunoglobulin terminals on the outside and the kinase areas on the inside.



Now along comes a GAS6 ligand, and it attaches to and connects the MERTK molecules at the immunoglobulin ends and this activates the kinase tails inside the cell.



Once activated the kinase ends commence pathway activation via the phosphorylation process. The pathways are depicted below.



It is the activation of these pathways by the excess GAS6 production or the excess MERTK production or both that results in excess proliferation and metastasis.

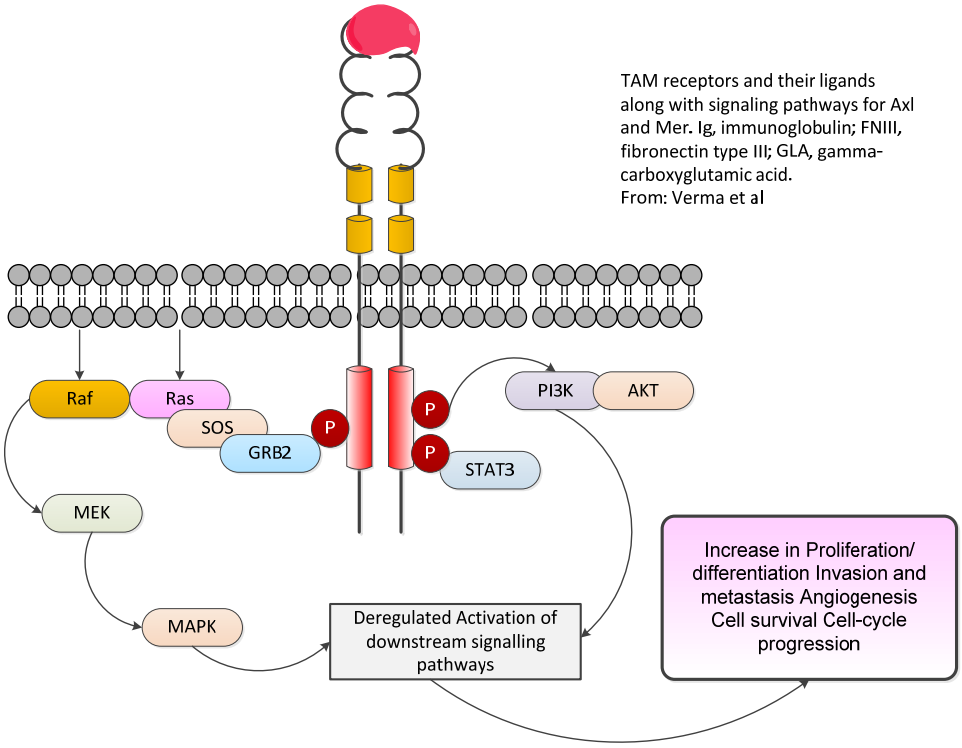
As Verma et al relate about the pathway:

Studies using chimeric Mer receptors expressed in NIH3T3 fibroblasts linked downstream signaling pathways, such as PI3K, phospholipase C-g (PLCg), and ERK, to Mer activation. Gas 6–dependent activation of Mer stimulates phosphorylation of ERK1/2, leading to cellular transformation and increased proliferation and DNA synthesis.

The ultimate downstream targets of the pathway differ according to cell type and tissue microenvironment. In leukemia cells, ligand-dependent activation of EGF receptor (EGFR)–Mer chimeric receptor stimulates phosphorylation of Akt, ERK 1/2, and p38 mitogenactivated protein kinases (MAPK), which results in decreased apoptosis but no change in proliferation (30). Expression of CD8-Mer chimera in pro-B cells results in transcriptional activation of NF-kB via PI3K/Akt.

Additional activation of p38/MAPK and meiosis-specific serine/threonine protein kinase 1 (MEK1) occurs via CD8-Mer, leading to protection from apoptosis. Some atypical signaling pathways involved in cell survival have been studied as a link between Mer and the actin cytoskeleton via growth factor receptor-bound protein 2 (Grb2), Shc, and Vav1. Downregulation of the proapoptotic tumor suppressor WW domain-containing

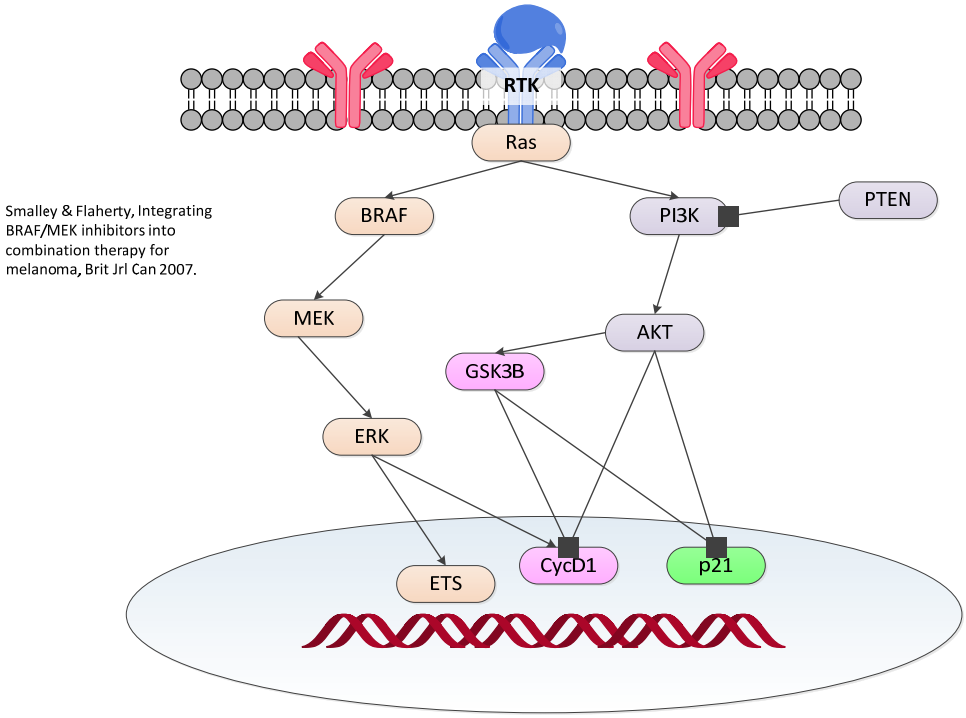
We depict below how one can inhibit this process. We depict an inhibitor molecule which binds to the sites as before but now does not activate the TK pathways. The inhibitor must be stronger in affinity than the GAS6 which most likely is still in ECM abundance.



Note above the RAs to RAF (especially BRAF) to MEK to MAPK pathways flow. We have examined this in details elsewhere[4]. The implication is that by targeting the TK Receptor, one targets all elements of the pathway. It should be noted however that the separate pathway elements may be activated and over expressed via other factors such as epigenetic ones. Thus the suggestions of Schlegel et al are of great merit but should be balanced by understanding the epigenetic issues as well.

An example of a pathway and its control with BRAF functionality is depicted below:

[4] See McGarty, Melanoma Genomics, DRAFT, 2013.



This simple explanation is also a paradigm for many other such pathway activations and especially for those of the tyrosine kinase verity.

3 MER AND MIRNA

There are other dimensions of interest here as well. In cancers there unfortunately is not just a single point of failure. There often are multiple. We show here just another example where MER and miRNA play an interesting role. This is an essential point to make because all too often the initial observers may all too often jump at a simple solution leaving behind a complexity of other factors which take control.

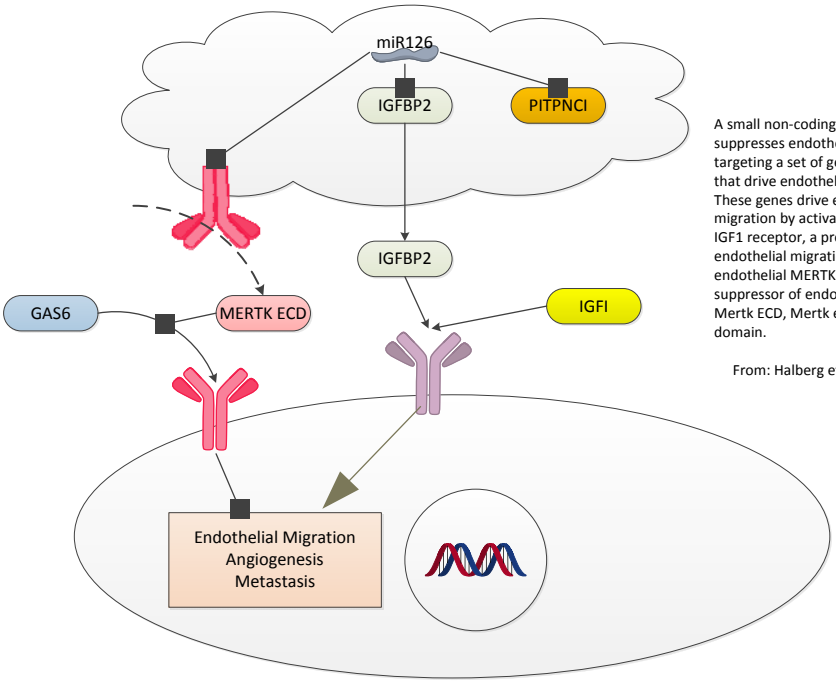
Let us consider a miRNA control using MER. As Halberg et al state:

Tumours require the establishment of vasculature for their increasing nutrient, energy, and oxygen requirements as well as for removal of metabolic waste. Cancer cells within a tumour generate such pathologic vasculature by recruiting endothelial cells to the tumour site. This is accomplished by secreting molecular factors, such as the well-known vascular endothelial growth factor (VEGF, into the extracellular space.

VEGF binding to VEGF receptors on endothelial cells results in the migration and recruitment of endothelial cells. In this way, proteins expressed by cancer cells can regulate the cellular and structural content of tumours—giving rise to continued tumour growth. Recent work has revealed a major role for another class of genes— known as small non-coding RNAs (microRNAs)—in the regulation of endothelial recruitment and tumour angiogenesis.

One member of this family (miR-126) was recently found to inhibit endothelial recruitment by suppressing a set of cancer genes that activate endothelial migration. In this way, a non-coding RNA expressed by cancer cells could shape the tumour and metastatic microenvironment.

This is thus depicted below from the Halberg paper. Here we have two cells, the top cell is a cancer cell where miR126 is blocking IGBP2 and blocking the MERTK receptor which in turn would have blocked the entrance of GAS6. But since miR126 has blocked the blocker, we have excess GAS6. Thus we have a problem, namely the GAS6 "overproduction" is really a failure to block resulting from the cancer cell miR126 production.



A small non-coding RNA, miR-126, suppresses endothelial migration by targeting a set of genes in cancer cells that drive endothelial migration. These genes drive endothelial migration by activating endothelial IGF1 receptor, a promoter of endothelial migration, and inhibiting endothelial MERTK receptor, a suppressor of endothelial migration. Mertk ECD, Mertk extracellular domain.

From: Halberg et al, EMBO 2012

It is critical always therefore to look across all paths, direct as well as epigenetic.

As Zhuang et al state:

Angiogenesis plays a crucial role during tumorigenesis and much progress has been recently made in elucidating the role of VEGF and other growth factors in the regulation of angiogenesis. Recently, microRNAs (miRNAs) have been shown to modulate a variety of physiological and pathological processes.

We identified a set of differentially expressed miRNAs in microvascular endothelial cells co-cultured with tumour cells. Unexpectedly, most miRNAs were derived from tumour cells, packaged into microvesicles (MVs), and then directly delivered to endothelial cells.

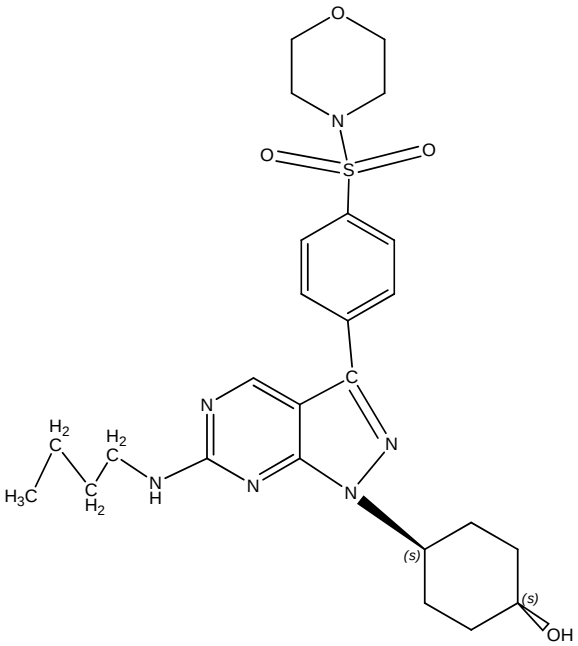
Among these miRNAs, we focused on miR-9 due to the strong morphological changes induced in cultured endothelial cells. We found that exogenous miR-9 effectively reduced SOCS5 levels, leading to activated JAK-STAT pathway. This signalling cascade promoted endothelial cell migration and tumour angiogenesis.

Remarkably, administration of anti-miR-9 or JAK inhibitors suppressed MV-induced cell migration in vitro and decreased tumour burden in vivo. Collectively, these observations suggest that tumour-secreted miRNAs participate in intercellular communication and function as a novel pro-angiogenic mechanism.

4 INHIBITORS

Inhibitors of pathways are being developed at a rapid rate. Knowing the pathway and molecular structure of the receptors it is somewhat readily possible to develop a strong inhibitor, a molecule that interferes with the normal ligand.

Schlegel et al have developed and tested an inhibitor of the MERTK receptor and it is shown below.



Schlegel et al characterize this molecule as follows:

A novel MERTK tyrosine kinase inhibitor, UNC1062, inhibits MERTK mediated signaling, promotes apoptosis, and inhibits colony formation in melanoma cells. While activating mutations in BRAF and NRAS occur in melanoma at rates of 41% and 18%, respectively, lower mutation frequency or gene amplifications in other signaling molecules, such as RTKs, can also contribute to melanoma pathogenesis.

UNC1062 was developed as a MERTK-selective tyrosine kinase inhibitor. Its structure is based on a previously published pyrazolopyrimidine scaffold, and it has an improved affinity and specificity profile compared with its parent compound, UNC569 .

UNC1062 potently inhibits MERTK kinase activity in vitro and exhibits specificity within the TAM family. Treatment of HMCB and G361 cells with increasing concentrations of UNC1062 resulted in a potent dose-dependent reduction in MERTK phosphorylation

In the work of Verma et al they present an interesting collection of molecules which exhibit inhibitor characteristics (see their Figure 2). This is an expansion of what Schlegel et al have presented.

Again from Schlegel et al we have:

MAPK/ERK and PI3K/AKT are 2 of the most frequently dysregulated pathways in melanoma. These 2 pathways not only play a role in melanoma development and progression, but are also involved in primary and secondary resistance to BRAF inhibitors.

The observation that MERTK signals via both pathways, as well as through others whose roles in melanoma biology are currently unclear (e.g., STAT6), not only highlights the complex regulation of these pathways by membrane receptors, such as MERTK, but may also provide a therapeutic advantage, since targeting MERTK may disrupt signaling in multiple pathways.

These observations and the data presented here suggest that MERTK-targeted therapies could potentially be considered for patients, irrespective of BRAF and NRAS status and/or prior treatment with BRAF inhibitors.

The latter observation is of possible significant merit. Namely the MERTK targeting allows for alternative pathway blocking, namely doing so at the source of pathway activation.

5 OBSERVATIONS

We conclude with some general and specific observations. This work by Schlegel et al is of significant importance for reasons already indicated.

1. **MERTK presents an attractive target for metastatic diseases.**

To best summarize, we use the words directly from the paper. Schlegel et al conclude:

We believe this work has led to several novel insights.

First, MERTK expression is significantly elevated in distant metastatic tumors compared with primary melanomas.

Second, MERTK is overexpressed in approximately half of melanoma cell lines, irrespective of BRAF and NRAS status, and is an active receptor.

Third, targeting MERTK suppresses prosurvival pathways such as STAT6, AKT, and ERK1/2.

Fourth, targeting MERTK suppresses colony-forming potential and migration.

And fifth, targeting MERTK in vivo retards tumor growth in a human melanoma xenograft model.

The finding that MERTK expression is highest in distant metastatic melanomas compared with primary melanomas and the roles of MERTK in colony formation, migration, and invasion suggest that MERTK plays a role in the progression of primary melanomas and the development of distant metastases.

Similar to the observations in this report, the migratory nature of glioblastoma cells could be reduced by MERTK inhibition with either shRNA knockdown or a MERTK monoclonal antibody, suggesting that increased MERTK expression may contribute to outgrowth of the metastatic tumor.

2. **MER and other TAM receptors show significant impact across broad areas of cancer activity.**

Now Verma et al present an interesting summary table as show below which recounts what cancer types are also upregulated TAM pathways. The breath of such upregulation is significant. It also may present significant opportunities for blockage molecules, namely inhibitors of the total pathway.

Cancer type	Upregulation of Axl/Mer/Gas
Acute leukemia (ALL, AML)	Axl, Mer
Astrocytoma	Axl, Mer, Gas 6
Breast cancer	Axl, Mer, Gas 6
Colorectal carcinoma	Axl
Esophageal adenocarcinoma	Axl
Gastrointestinal stromal tumors	Axl
Gastric cancer	Axl, Mer, Gas 6
Hepatocellular carcinoma	Axl
Kaposi sarcoma	Axl
Lung cancer	Axl
Mantle cell lymphoma	Mer
Melanoma	Axl, Mer
Ovarian cancer	Axl, Gas 6

Osteosarcoma	Axl
Pancreatic ductal adenocarcinoma	Axl, Gas 6
Renal cell carcinoma	Axl, Gas 6
Prostate cancer	Axl, Mer
Thyroid cancer	Axl, Gas 6
Uterine endometrial cancer	Axl, Gas 6

3. **GAS6 Inhibition on MERTK by inhibitors is an attractive approach to metastatic melanoma**

Developing receptor inhibitors is a powerful approach to controlling metastatic growth and proliferation.

As Verma et al state:

A potential ability of sAxl to serve as a natural antagonist of Gas 6 could have clinical relevance. Similarly, the membrane-bound Mer protein is cleaved in the extracellular domain via a metalloproteinase (38). Further studies are needed to establish sAxl and sMer as important biomarkers for correlation with disease stage and predicting prognosis.

As Segal et al state:

A subset of genes, including the small monomeric GTPase RABB33, the proto-oncogene MERTK, the glycopeptide hormone STC1, and the neuropeptide GAL were shown to discriminate CCS/MSP from both STS and melanoma.

We further surveyed specific genes of interest and found melanoma differentiation antigens TYRP1, TYRP2/DCT, and MART-1 to be expressed at varying levels in the CCS/MSP specimens. PMEL17 was most consistently expressed in all four tumors in a similar distribution to that of MITF. Interestingly, SOX10, which induces MITF expression, was expressed in all CCS/MSP and most melanoma specimens

Thus it appears that this approach and ones like it are useful for thorough examination as attractive and effective means of metastatic control and management.

However there is still a long way from this point to approved therapeutics.

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