REVIEW

Antiangiogenic therapies for pheochromocytoma and paraganglioma

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Abstract

Metastatic pheochromocytomas and paragangliomas are rare, highly vascular tumors that spread primarily to the lymph nodes, skeletal tissue, lungs, and liver. Tumor morbidity is related to their size, location, hormonal activity, vascular nature, and rate of progression. Systemic therapies for this indication are limited. Only high-specific-activity iodine-131 metaiodobenzylguanidine is approved in the United States for treatment of these patients, and not all patients are candidates for this radiopharmaceutical. Antiangiogenic medications are currently being evaluated in prospective clinical trials for patients with metastatic pheochromocytomas and paragangliomas, and preliminary results have been encouraging. Antiangiogenic medications frequently offer antineoplastic effects with sometimes durable responses. However, cardiovascular toxicity and the development of tumor resistance may limit their efficacy. Experience derived from clinical trials is being used to identify mechanisms to effectively improve drug toxicity and possibly prevent the emergence of resistance. Therefore, antiangiogenic medications represent a therapeutic option for patients with metastatic pheochromocytomas and paragangliomas. Furthermore, in the world of oncology, there is strong scientific interest in the development of clinical trials that combine antiangiogenic medications with other modalities such as immunotherapy, radiopharmaceuticals, and hypoxia inhibitors since these combinations may substantially enhance clinical outcomes, including survivorship. In this review, we examine the progress made to date on antiangiogenic treatments for patients with metastatic pheochromocytomas and paragangliomas.

Key Words

- ▶ pheochromocytoma/paraganglioma
- ▶ succinate dehydrogenase subunit B
- ▶ angiogenesis
- ▶ systemic therapy
- ▶ tyrosine kinase inhibitors
- ▶ objective response rate
- ▶ progression free survival
- ▶ toxicity

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Introduction

Pheochromocytomas and paragangliomas are neuroendocrine tumors that make up less than 1% of all endocrine neoplasias. Pheochromocytomas and paragangliomas arise from the adrenal medulla and the extra-adrenal paraganglia, respectively (Lam 2017). Pheochromocytomas are sympathetic tumors that commonly release catecholamines such as noradrenaline and/or adrenaline. Paragangliomas are

classified as parasympathetic or sympathetic, depending on the autonomic nervous system ganglia of origin. Parasympathetic paragangliomas are located primarily in the head and neck and do not release catecholamines. These tumors are frequently asymptomatic and are discovered during routine physical examination or accidentally by imaging studies. Sympathetic paragangliomas are located in the thorax, abdomen, and pelvis and frequently release

excessive amounts of noradrenaline (Lam 2017). Unlike parasympathetic paragangliomas, pheochromocytomas and sympathetic paragangliomas predispose to hormonal symptoms such as hypertension, palpitations, sweats, headaches, and diabetes mellitus. These symptoms are not specific, and the diagnosis is frequently overlooked. A high index of suspicion is essential to discover these tumors (Manger 2009).

Most pheochromocytomas and paragangliomas are curable with surgery (Lenders *et al.* 2014). However, 15–20% of these tumors are metastatic. Unfortunately, there is no histological, molecular, biochemical, or genetic predictor of the tumor's metastatic potential. The World Health Organization (WHO), however, recognizes that all pheochromocytomas and paragangliomas have the potential to spread distantly and recommends that the term 'malignant' be avoided in this setting, for example, 'metastatic tumor' is preferred to 'malignant tumor' (Lam 2017). Most patients with metastatic tumors present with advanced disease, and their lifespan is usually compromised. Metastases happen in less than 10% of parasympathetic paragangliomas, in up to 25% of pheochromocytomas, and in 40–70% of sympathetic paragangliomas (Ayala-Ramirez *et al.* 2011, Hamidi *et al.* 2017, Jasim & Jimenez 2019). Metastatic pheochromocytomas and paragangliomas (MPPGs) spread mainly to the lymph nodes, skeletal tissue, lungs, and liver and exhibit variable aggressiveness. Metastases may be present at the time of diagnosis or may become apparent after the discovery of the primary tumor (Jimenez *et al.* 2013, Roman-Gonzalez & Jimenez 2017). Patients with MPPGs frequently exhibit a large tumor burden with overwhelming symptoms of catecholamine excess. Difficult to control hypertension, acute and chronic cardiovascular disease, severe constipation, skeletal-related events, liver insufficiency, and respiratory distress are some of the complications of MPPGs (Ayala-Ramirez *et al.* 2013, Thosani *et al.* 2015, Roman-Gonzalez *et al.* 2018, Jasim & Jimenez 2019). Systemic therapies are limited; in the United States, only high-specific-activity iodine-131 metaiodobenzylguanidine (HSA-I-131-MIBG) has been approved for MPPGs that express the noradrenaline transporter (Pryma *et al.* 2019).

Up to 50% of patients with MPPGs carry an inactivating germline mutation of the succinate dehydrogenase subunit B (*SDHB*) gene (Dahia 2014). These mutations predispose to pseudohypoxia, inflammation, necrosis, abnormal cell proliferation, angiogenesis,

DNA hypermethylation, and epithelial-to-mesenchymal transition (Favier *et al.* 2015). This molecular phenotype is common to other hereditary and apparently sporadic MPPGs (Dahia *et al.* 2005). As in many other cancers, pseudohypoxia and angiogenesis could be therapeutic targets. In this review, we focus on angiogenesis as a hallmark of MPPGs, the clinical implications in MPPGs, and the potential benefits of antiangiogenic therapies.

Angiogenesis as a hallmark of cancer

Development of a metastatic tumor is a complex event that involves several complementary biological capabilities known collectively as the 'hallmarks of cancer'. These hallmarks include abnormal and sustained proliferative signaling, enabling of replicative immortality, genomic instability and mutation, deregulation of cellular energetics, tumor-promoting inflammation, angiogenesis, evasion of growth suppressors, resistance to cell death, activation of invasion and metastasis, and avoidance of immune recognition and destruction (Hanahan & Weinberg 2011). As the tumor develops, its microenvironment changes; this microenvironment is very complex and dynamic and includes non-cancer cells such as endothelial cells, pericytes, immune inflammatory cells, fibroblasts, and an extracellular matrix that is constantly built and remodeled. The interactions between the cancer and non-cancer cells and the extracellular matrix collectively enable the tumor's growth and spread (Hanahan & Weinberg 2011).

From the perspective of the hallmarks of cancer, MPPGs have a lower mutation burden and proliferative rates and less inflammation than other malignancies. MPPGs are, however, characterized by a pronounced angiogenesis.

Angiogenesis suggests the formation of new capillaries from previous vessels. Under normal conditions, angiogenesis determines organ growth, wound repair, reproduction, tissue differentiation and communication, and embryonic development. Angiogenesis is tightly regulated by intracellular oxygen tension and a balance between proangiogenic and antiangiogenic factors (Pugh & Ratcliffe 2003). The endothelial and smooth muscle cells sense oxygen concentrations with use of various mechanisms including endothelial nitric oxide synthase, reduced NAD phosphate (NADPH) oxydases, heme oxygenases, and others. Interactions of oxygen

sensors with the family of transcription factors called hypoxia-inducible factor alpha subunit (HIF- α) determine the cellular adaptation to the oxygen tension. With adequate oxygen concentrations, HIF- α have a short life due to hydroxylation by prolyl hydroxylases and binding to von Hippel-Lindau (VHL) protein that leads to ubiquitination and proteasome degradation. When oxygen concentrations are low, HIF- α are stabilized. In the nuclei, HIF- α transcribe the genes of several proangiogenic and cell growth factors including vascular endothelial growth factors (VEGFs) A, B, C, D, and E; fibroblast, platelet-derived, hepatocyte, and epidermal growth factors; transforming growth factors- α and β ; tumor necrosis factor- α (TNF- α); and others. These factors induce cell replication and angiogenesis. Once the energetic demands are satisfied, antiangiogenic factors are released, preventing additional vascular formation and removing the newly created vessels. Inhibitors of angiogenesis include angiostatin, endostatin, vasostatin, osteopontin, and kininogen domains (Sakurai & Kudo 2011).

From an oncological perspective, angiogenesis is critical for the development of cancer cells and metastatic colonization (Folkman 1971). Tumor replication requires angiogenesis to supply oxygen and nutrients and eliminate toxic products. Without a blood supply, the tumor cannot develop beyond the limit of diffusion (no more than a few millimeters) and therefore remains small. Due to replication, the new and existing tumor cells lack nutrients and oxygen, stabilizing the HIF- α ; in the nucleus, the HIF- α bind to hypoxia response elements with downstream transcription of proangiogenic molecules (Pugh & Ratcliffe 2003). At the same time, antiangiogenic molecules are inhibited, switching the tumor's phenotype from non-angiogenic to angiogenic (Hanahan & Folkman 1996). VEGFs interact with peritumoral endothelial and cancer cell membrane receptors, activating the MAPK pathway and inducing angiogenesis and tumor progression (Pugh & Ratcliffe 2003). The new vessels do not look like normal vessels. These vessels are disorganized and enlarged, and their endothelial cells are not well-connected, favoring tumor expansion, vessel invasion, tumor cell separation, and cell spread (Lugano *et al.* 2020).

Gaining an understanding of tumor hypoxia and angiogenesis has had a huge therapeutic impact in cancer. Antiangiogenic therapies such as axitinib, bevacizumab, cabozantinib, lenvatinib, pazopanib, sorafenib, or sunitinib have been approved treatments for several malignancies (Lugano *et al.* 2020).

Angiogenesis in pheochromocytomas and paragangliomas

Imaging and surgical descriptions indicate that most pheochromocytomas and paragangliomas are highly vascular tumors. Conventional imaging studies such as CT and MRI frequently describe a rich and complex peritumoral network of vessels, especially in large tumors; furthermore, these tumors are described as bright lesions in MRI T2-weighted sequences (Varghese *et al.* 1997). Surgical interventions require careful planning and sometimes the support of a vascular surgeon. When compared with resection of adrenal adenomas, resection of pheochromocytomas is more frequently associated with vascular complications, bleeding, blood transfusions, and longer and more expensive hospital admissions (Parikh *et al.* 2017).

In the early 1990s, murine and human pheochromocytomas were found to express VEGFs (Claffey *et al.* 1992, Sheehy *et al.* 1997). In addition, the rat PC12 pheochromocytoma cell metastatic model exhibited peritumoral angiogenesis mediated by VEGFs and inhibited by anti-VEGF antibodies (Middeke *et al.* 2002). These observations led to the hypothesis that angiogenesis may predict the metastatic potential of pheochromocytoma and paraganglioma and that antagonizing the VEGF pathway may improve clinical outcomes.

Liu *et al.* correlated the expression of factor VIII/von Willebrand factor antigen with the tumor's microvessel count and density and the presence of capsular/vascular invasion and/or metastases (Liu *et al.* 1996). The study included 24 pheochromocytomas and 18 paragangliomas. Six tumors had capsular/vascular invasion and four had metastases. The study found that the highest vascular density was in the tumor periphery and that MPPGs and tumors with capsular/vascular invasion had a significantly higher number of blood vessels when compared with tumors without capsular/vascular invasion and metastases. Of interest, there was no difference in vascular content between pheochromocytomas and paragangliomas. The study had limitations; however, the number of samples for analysis was small; there was variability in the number of blood vessels within histologic sections; and the staining for factor VIII/von Willebrand factor antigen likely missed small vessels. Furthermore, the follow-up of these patients was short, and long-term outcomes with regard to metastases and survival were unknown.

Ohji *et al.* correlated the expression of CD34 with the tumor's microvessel count (Ohji *et al.* 2001).

CD34 is a more sensitive endothelial marker than the factor VIII/von Willebrand factor antigen is. The study included 23 pheochromocytomas and 2 paragangliomas. Four patients had metastases. The study confirmed angiogenesis; however, the microvascular counting was not different between MPPGs and tumors without metastases.

Zielke *et al.* tried to understand the association between angiogenesis and MPPG progression (Zielke *et al.* 2002). Their study included ten tumors with no metastases, five tumors with capsular/vascular invasion, and five MPPGs. The results indicated that the vascular surface density cannot predict metastatic potential. Nevertheless, concentrations of VEGFs were significantly higher in MPPGs. As in previous studies, the sample for analysis was small.

In a larger study by Salmenkivi *et al.*, the expression of VEGFs was quantified in 69 pheochromocytomas and 36 paragangliomas (Salmenkivi *et al.* 2003). Their study included control samples of normal adrenal medulla, 60 tumors with no metastases or peritumoral invasion, 37 tumors with capsular/vascular invasion but no metastases, and 8 MPPGs. The study found no expression of VEGFs in normal adrenal medulla cells or in 20 tumors without metastases. Nevertheless, the majority of pheochromocytomas and paragangliomas without distant metastases ($n=77$) had immunoreactivity for VEGF. There was moderate to high expression of VEGF in several tumors without metastases and in all MPPGs. Other studies have noticed that expression of VEGFR 1–2 is present in pheochromocytomas and paragangliomas but not in the normal adrenal medulla and that levels are, in general, higher in MPPGs than in tumors without metastases (Takekoshi *et al.* 2004, Ferreira *et al.* 2014).

The vascular architecture also has been evaluated as a potential predictor of metastases (Favier *et al.* 2002). In this study, tumors that did not have metastases had short, straight, vascular segments with uniform distribution. Conversely, MPPGs had larger segments of irregular length and displayed at least one branching on open luminal space. Expression of proangiogenic factors such as EPAS1, VEGF, VEGFR1–2, and endothelin receptors A (ETA) and B (ETB) was more obvious in MPPGs; ETA in pericytes and tumoral cells and ETB in endothelial cells discriminated between tumors with and without metastases (Favier *et al.* 2002, Oudijk *et al.* 2015). However, the sensitivity and specificity rates of the irregular vascular architecture as a predictor of metastases were only 59.7% and 72.9%, respectively; therefore, an abnormal vascular architecture cannot

be used as a single predictor of metastases (Bialas *et al.* 2014, Oudijk *et al.* 2015).

CD31 and CD105 are sensitive endothelial markers with high expression in the central areas of MPPGs where oxygen tension is expected to be low. The evaluation of these markers did not find differences in vascular density between MPPGs and tumors without metastases (Bialas *et al.* 2014). Vascular density cannot be considered a strong predictor of metastases (Rooijens *et al.* 2004). Together, these studies indicate that pheochromocytomas and paragangliomas exhibit increased and abnormal angiogenesis that is, in part, mediated by the VEGF pathway; however, angiogenesis *per se* cannot be used to predict the metastatic potential of these tumors. None of these studies correlated angiogenesis with the tumor's genetic background.

Angiogenesis is influenced by the genetic background. Mutations of the *SDHB* gene are prevalent in patients with MPPGs. *SDHB* codifies for the subunit B of the mitochondrial enzymatic complex 2 or succinate dehydrogenase. This enzymatic complex allows for the conversion of succinate into fumarate in the citric acid cycle and for the transport of electrons through the internal mitochondrial membrane (Mannelli *et al.* 2015). Thus, succinate dehydrogenase is a regulator of the cellular metabolism of oxygen. Inactivating mutations of *SDHB* leads to pseudohypoxia and accumulation of succinate (Gimenez-Roqueplo *et al.* 2002); succinate inhibits prolyl hydroxylases, stabilizing HIF- α and triggering abnormal cell growth and angiogenesis (Favier & Gimenez-Roqueplo 2010). In fact, *SDHB* pheochromocytomas and paragangliomas are frequently large, highly vascular tumors that express high concentrations of VEGFs, PDGF, endothelin, and angiopoietin (Gimenez-Roqueplo *et al.* 2002). Of interest, inactivating mutations in the genes that code for the subunits A, C, D, and the co-factor SDHAF2 of succinate dehydrogenase also cause pseudohypoxia and angiogenesis (Favier *et al.* 2002, 2012). This molecular phenotype is shared with *VHL*-related pheochromocytomas and paragangliomas and a substantial number of sporadic tumors (cluster 1) (Takekoshi *et al.* 2004, Favier & Gimenez-Roqueplo 2010, Favier *et al.* 2012, 2015). Cluster 1 tumors with and without metastases exhibit similar levels of VEGF and other proangiogenic factors. Angiogenesis cannot predict the cluster 1 metastatic potential. In fact, most tumors associated with *SDHA*, *SDHC*, *SDHD*, and *VHL* mutations are not metastatic.

Cluster 2 pheochromocytomas and paragangliomas are associated with activating mutations of the *RET*

proto-oncogene or inactivating mutations of the neurofibromatosis type 1 (*NF1*), *MAX*, *HRAS*, *MET*, *FGFR1*, or *TMEM127* genes (Crona *et al.* 2013, Castro-Vega *et al.* 2015, Toledo *et al.* 2016, Fishbein *et al.* 2017). Although cluster 2 tumors are not characterized by a cellular environment of pseudohypoxia and their expression of VEGFs is lower than that in cluster 1 tumors, cluster 2 tumors exhibit angiogenesis (Santoro *et al.* 2002). *RET*, *NF1*, and *RAS* mutations upregulate HIF- α through activation of extracellular signal regulated kinase (ERK) in the MAPK pathway (Richard *et al.* 1999, Lim *et al.* 2004). ERK phosphorylates HIF-1 α , inducing angiogenesis and tumor growth. *TMEM127* and *MAX* seem to upregulate HIF- α through activation of the mTOR pathway; however, the details of this mechanism are unclear (Brugarolas *et al.* 2004, Land & Tee 2007). Cluster 2 MPPGs seem to have higher concentrations of VEGFs compared with levels in tumors without metastases (Favier *et al.* 2012). Recently, the TCGA identified a third cluster of pheochromocytomas and paragangliomas (Fishbein *et al.* 2017). This cluster represents ~5% of these tumors and is associated with Wnt pathway abnormalities. These tumors may have a metastatic phenotype. The mechanism of angiogenesis in these tumors is currently unknown.

Several other biomarkers associated with a high risk of metastases, regardless of tumor *SDHB* status, have been identified. These biomarkers include somatic mutations of *ATRX* and telomerase reverse transcriptase (*TERT*), high *TERT* expression, and miRNA cluster 182/96/183, miR-210, and miR-483 (Calsina *et al.* 2019, Goncalves *et al.* 2019, Job *et al.* 2019, Dahia *et al.* 2020). Of interest, some of these biomarkers promote angiogenesis. For example, aberrant expression of *TERT*, in addition to causing tumor immortalization, increases expression of VEGFs by interacting with transcription factor Sp1 (Liu *et al.* 2016); also, miR-210 is involved in vascular remodeling in the tumor microenvironment (Jung *et al.* 2017).

Antiangiogenic therapy for MPPGs

Axitinib, cabozantinib, lenvatinib, pazopanib, and sunitinib are antiangiogenic medications currently being evaluated in clinical trials for patients with MPPGs. These medications inhibit multiple tyrosine kinase receptors involved in angiogenesis, tumor growth, invasiveness, and the development of metastases (Table 1). Used as antiangiogenic therapies, they prevent activation of the VEGF pathway, leading to vascular depletion, normalization of the tumor vascular network, and enhanced recognition of tumor cells by the immune system (Fig. 1) (Shrimali *et al.* 2010, Carmeliet & Jain 2011, Goel *et al.* 2011, Lugano *et al.* 2020). Since we do not have reliable models of human MPPGs, the physiological effects of antiangiogenic therapies are discussed by extrapolating information derived from important basic, translational, and clinical studies in other vascular malignancies treated with these drugs. However, we recognize that the actions of antiangiogenic therapies in MPPGs may be somewhat different or even unique compared with these actions in other cancers or among clusters/subtypes of MPPGs.

Vascular depletion decreases the supply of oxygen and nutrients to cancer cells, leading to their death. Normalization of the tumor vascular network suggests restoration of the normal structure and function of the tumor vessels. Thus, the tumor vessels' diameter decreases; adhesion of endothelial cells increases, limiting vascular permeability; and delivery of oxygen to the tumor cells increases, decreasing the rate of tumor growth and spread. Furthermore, normalization of the vasculature may facilitate the delivery of concomitant therapies such as radiotherapy, chemotherapy, and/or immunotherapy (Fig. 1). This has been demonstrated in mouse models of several cancers other than MPPGs and may explain the significantly longer progression-free survival (PFS) rates observed in some cancer patients treated with a combination of antiangiogenic drugs and

Table 1 Targets and affinity of tyrosine kinase receptor inhibitors currently being evaluated in metastatic pheochromocytoma and paraganglioma.

Drug	IC50 (nm)						
	VEGFR-1	VEGFR-2	VEGFR-3	PDGFR- β	RET	c-MET	FGFR1
Axitinib	1.2	0.25	0.29	1.6	-	-	-
Cabozantinib	-	0.035	-	-	4	1.3	-
Lenvatinib	22	4	5.2	39	-	-	46
Pazopanib	10	30	47	84	-	-	74
Sunitinib	2	9	17	2	41	4000	-

Figure 1

Vascular consequences of anti-angiogenic therapy. Antiangiogenic therapy may lead to (A) vessel depletion and (B) normalization of tumor vascular network with enhanced recognition by the immune system. The consequences of these effects and their therapeutic implications are different. RBC: red blood cells; TAM: tumor-associated macrophages; DC: dendritic cells; MDSC: myeloid derived suppressor cells.

chemotherapy compared with rates associated with use of chemotherapy alone (Mazzone *et al.* 2009, Jayson *et al.* 2012). However, normalization of the tumor vascular function may not happen in every cancer treated with antiangiogenic therapy. In patients with glioblastoma, for example, bevacizumab induces morphological vascular normalization, but vascular function is not improved; this lack of functional improvement leads to decreased tumor blood flow, hypoxia, tumor growth and invasion, and no benefit in survivorship (Obad *et al.* 2018). Whether morphological and functional vascular normalization could happen in MPPGs treated with antiangiogenic therapy still needs to be determined.

Regarding tumor angiogenesis and its impact on the immune system, overexposure to angiogenic factors

decreases expression of endothelial cells' adhesion molecules, impairing the activation of inflammatory pathways mediated by factors such as interleukin-1, TNF- α , and others that would otherwise lead to immune system recognition (Griffioen *et al.* 1996); furthermore, the activation of the VEGF prevents dendritic cell maturation (antigen presenting cells), discourages T cell-infiltration, and promotes the activation of regulatory T cells that also prevent the recognition of the cancer cells by the immune system (Manegold *et al.* 2017); this mechanism perhaps explains, at least in part, why the cells of vascular tumors are not recognized by the immune system. The vascular normalization that follows antiangiogenic therapy may increase the expression of adhesion molecules that attract cytotoxic T cells and decrease the presence of

immune-suppressive T cells (Fig. 1) (Shrimali *et al.* 2010). In addition to the antiangiogenic properties of this therapy, blockage of the VEGF pathway, together with inhibition of other tyrosine kinase receptors, such as fibroblast growth factors (lenvatinib) or c-met receptors (cabozantinib), may prevent tumor cell replication and/or tumor invasiveness and metastases (Trusolino *et al.* 2010). As such, these drugs target multiple fronts and are promising therapies for patients with MPPGs.

These drugs have been tested in phase 1, 2, and 3 clinical trials for malignancies other than MPPGs. Subsequently, their pharmacokinetics, pharmacodynamics, oncological benefits, and safety profile have been, in general, well-characterized (Tables 1 and 2). Nevertheless, their impact on MPPGs is for most part still to be determined. The following is a discussion of the potential benefits and adverse effects of these medications in patients with MPPGs based on clinical observations derived from case reports, retrospective studies, and clinical trials (Table 3). The descriptions follow the historical order of drug discovery.

Sunitinib

Sunitinib inhibits VEGFRs 1–3, PDGFRs, RET, FLT3, and Kit. receptors. The drug is approved for the treatment of clear cell renal cell carcinoma, pancreatic neuroendocrine tumors, and gastrointestinal stromal tumors. Sunitinib is prescribed in cycles of 50 mg daily for 4 weeks followed by a 2-week rest period. Sunitinib is the first antiangiogenic medication recognized as a potential treatment for MPPGs.

Preclinical studies with sunitinib in a PC12 xenograft mouse model demonstrated potent antiangiogenic activity. Inhibition of VEGFRs by sunitinib prevented blood vessel sprouting and decreased the number and extension of the tumor microvessels; furthermore, sunitinib caused apoptosis in the PC12 cells (Saito *et al.* 2012, Denorme *et al.* 2014). PC12 apoptosis was mediated by inactivation of antiapoptotic molecule Bcl-2 and activation of the proapoptotic molecule BAD (Saito *et al.* 2012). Inhibition of angiogenesis and tumor

apoptosis led to a dramatic reduction in tumor size (Denorme *et al.* 2014). However, abrupt interruption of sunitinib led to rapid regrowth of the tumor and its vessels; this finding suggested that treatment with sunitinib must be continuous in order to preserve its therapeutic effect (Denorme *et al.* 2014). Of interest, in PC12 cells, the inhibition of VEGFR-2 by sunitinib decreased the activity of tyrosine hydroxylase, reducing the synthesis of catecholamines (Aita *et al.* 2012).

A recent, elegant study in a murine model of *SDHB* -/- paraganglioma combined three imaging modalities to explore the effects of sunitinib on tumor proliferation (CT), angiogenesis (Ultrafast Doppler ultrasound), and glucose metabolism (FDG-PET) (Facchin *et al.* 2020). The study noted that all mice treated with sunitinib had a decreased tumor size and glucose uptake, with some reduction in the length and volume of the tumor vessels – the effect of sunitinib in the tumor capillaries and other small vessels was not evaluable due to technical limitations. The effects of sunitinib on these three hallmarks were transitory and the tumor later developed resistance that inversely correlated with the inhibitory effects on the tumor vessels length and glycolysis. The results of this study suggested that metabolic symbiosis is a mechanism of resistance to sunitinib (discussed later). The study raised interesting questions on the use of complementary imaging studies in humans to better understand the actions of antiangiogenic medications. In addition, it suggested exploring therapeutic combinations (e.g. antiangiogenic therapies and antimetabolites) to delay/prevent tumor resistance.

One of the original reports in humans described the effects of sunitinib in a VHL patient with bilateral kidney cancers, multiple pancreatic neuroendocrine tumors, and a metastatic sympathetic paraganglioma with high expression of VEGF and PDGFR- β (Jimenez *et al.* 2009). The presence of angiogenesis in the paraganglioma and the multifocal kidney cancer supported the use of sunitinib. The patient exhibited impressive partial responses (PRs) in all tumors, improvement of hypertension, relief of pain related to bone metastases, and disease stabilization for some time. This study supported the development of a

Table 2 Pharmacokinetic properties of antiangiogenic therapies for MPPGs.

Drug	Bioavailability	Peak serum (hours)	Protein binding	Half-life (hours)	Hepatic metabolism	Excretion
Axitinib	58%	2.5–4	>99%	2.5–6.1	CYP3A4	Feces 41%, Urine 23%
Cabozantinib	Unknown	2–5	99.7%	55	CYP3A4	Feces 54%, Urine 27%
Lenvatinib	85%	1–4	98–99%	28	CYP3A4	Feces 65%, Urine 25%
Pazopanib	14–39%	2–4	>99%	31	CYP3A4	Feces >90%, Urine <4%
Sunitinib	Unknown	6–12	95%	40–60	CYP3A4	Feces 61%, Urine 16%

Table 3 Clinical trials of anti-angiogenic therapies in pheochromocytoma and paraganglioma.

Drug	Clinical trial identifier	Main center	Primary outcome	Translational studies	n	Design	Status on ClinicalTrials.gov
Axitinib	NCT03839498	Columbia University	Objective response rate	Activation of VEGFR pathway and relationship with response to therapy.	25	Single group assignment	Recruiting
Axitinib	NCT01967576	National Cancer Institute	Objective response rate	VEGFR in blood. Activation of VEGFR pathway and relationship with response to therapy.	14	Single group assignment	Active, not recruiting
Cabozantinib ^{ab}	NCT02302833	MD Anderson Cancer Center	Objective response rate	c-MET expression and relationship with prognosis and response to therapy Correlation between tumor response and germline/somatic mutational status.	22	Single group assignment	Recruiting
Lenvatinib	NCT03008369	Mayo Clinic	Objective response rate	Correlation between tumor response and germline/somatic mutational status	3	Single group assignment	Active, not recruiting
Pazopanib	NCT01340794	Mayo Clinic	Objective response rate	Correlation between tumor response and germline/somatic mutational status. Association of tumor response and expression of HIF-1 α , VEGFR and microvessel density	7	Single group assignment	Terminated (slow accrual) Results published
Sunitinib ^{ac}	NCT01371201	Gustave Roussy	PFS at 12 months	Effect on markers of biochemical activity	74	Parallel assignment	Recruiting
Sunitinib	NCT00843037	University Health Network	Clinical benefit rate	Predictors of response and surrogate markers of survival	25	Single group assignment	Active, not recruiting. Results published

Some trials were prematurely terminated due to low accrual and/or severe side effects such as hypertension.

^aThese are perhaps the most promising clinical trials; ^{ab}Preliminary results show DCR = 92%; ^{ac}The primary outcome of this trial is PFS at 12 months and it has a design that allows comparisons.

phase 2 trial for patients with VHL (Jonasch *et al.* 2011). This trial excluded patients with MPPGs because of safety concerns for blood pressure control.

In another report, sunitinib was discovered to be a potential treatment after it was prescribed to a patient with a nonresectable peri-renal paraganglioma that had been confused with kidney cancer (Joshua *et al.* 2009). The tumor decreased in size, which made its removal possible and later confirmed the diagnosis of paraganglioma. Two other patients with MPPGs were subsequently treated with sunitinib and achieved good oncological responses. One patient's tumor was biopsied 18 weeks after treatment; histological analysis revealed decreased expression of VEGFRs, and the patient responded to sunitinib for at least 40 weeks. Unfortunately, no other studies have

reported posttreatment biopsies; therefore, the ways in which antiangiogenic medications affect MPPGs' histology, vascular physiology, and microenvironment are unknown.

A retrospective intention-to-treat study of 17 patients treated with sunitinib showed clinical benefits in 47% of patients, including 3 patients who had a PR, disease stabilization for more than 2 years in some cases, normalization of hypertension, and discontinuation of antihypertensive medications in a few patients (Ayala-Ramirez *et al.* 2012). There were no complete responses (CRs). Patients with clinical benefits had tumors associated with *SDHB* mutations as well as apparently sporadic MPPGs; some of these patients had previously received chemotherapy or had been treated with I-131-MIBG.

The study showed a median PFS of 4.1 months and concluded that sunitinib may offer clinical benefits to some patients with MPPGs, irrespective of their genetic background. Nevertheless, adverse events due to excessive release of catecholamines because of tumor destruction as well as direct sunitinib-associated toxicity such as hypertension may prevent successful treatment. Therefore, patients with MPPGs treated with antiangiogenic medications must be treated with alpha- and beta-blockers and frequently with other antihypertensives before and during therapy; furthermore, supportive measures to prevent or treat adverse events directly related to the drug had to be implemented (i.e. moisturizers and hand and feet rest to prevent erythrodysesthesia and analgesic therapy to ameliorate or prevent pain exacerbation during tumor destruction). The study also suggested that a dose lower than 50 mg daily may provide clinical benefits with fewer adverse effects. As described in other cancers, tumor resistance was possible.

Bone metastases are quite common in patients with MPPGs (Ayala-Ramirez *et al.* 2013). In this retrospective study, patients with predominant bone metastases had a substantial reduction of glucose uptake in their FDG-PET scans (Ayala-Ramirez *et al.* 2012). A subsequent report suggested that sunitinib may decrease the rate of skeletal-associated events (Ayala-Ramirez *et al.* 2013).

A phase 2 clinical trial with sunitinib at 50 mg daily, 4 weeks on and 2 weeks off, was subsequently developed (SNIPP, NCT00843037) (O'Kane *et al.* 2019). The primary end point was disease control rate (DCR) at 12 weeks. The study included 23 patients with MPPGs and 2 patients with unresectable primary tumors without metastases. Three patients (13%) achieved PR, and 16 (70%) had stable disease (SD). Six patients with SD had tumor regression (12–27%). No patients had CR. The DCR was 83% (95% CI: 56–93%). PFS was 13.4 months (95% CI: 5.3–24.6 months). The most common adverse events (>60% of patients) were grade 1–2 fatigue, nausea/vomiting, and palmar-plantar erythrodysesthesia. Fifty-six percent of patients experienced grade 3 adverse events and 12% experienced grade 4 adverse events. Two patients had grade 3 or 4 hypertension. Several patients required dose reduction, and 20% of patients discontinued sunitinib because of adverse events including severe hypertension, myocardial infarction, and restrictive cardiomyopathy. The three patients with PR had hereditary MPPGs associated with *SDHA*, *SDHB*, and *RET* mutations, respectively. The patient with multiple endocrine neoplasia type 2 (MEN2) had the most impressive response, with a tumor size reduction

of 64% and stabilization for longer than 7 years. MEN2-MPPGs are quite rare (Thosani *et al.* 2013). Simultaneous inhibition of the RET and VEGF receptors may have led to this impressive response.

The SNIPP study showed that sunitinib benefits most patients with MPPGs. However, its antineoplastic actions are modest, with a low objective response rate (ORR). In addition, toxic effects with standard doses could be substantial and tumor resistance develops over time. Nevertheless, when compared with the PFS rates observed in clinical trials for patients with kidney cancer or pancreatic neuroendocrine tumors, the median duration of response in patients with MPPGs was actually good (11, 12, and 13.4 months, respectively) (Motzer *et al.* 2007, Faivre *et al.* 2017).

A phase 2 clinical trial comparing sunitinib at 37.5 mg daily vs placebo is ongoing (FIRSTMAPPP, NCT01371201) and may be able to provide survivorship data.

Pazopanib

Pazopanib inhibits VEGFRs 1–3, PDGFRs, c-FMS, and Kit receptors. The drug is approved for treatment of clear cell renal cell carcinoma and soft tissue sarcomas at a dose of 800 mg daily. Pazopanib became a drug of interest because its antiangiogenic mechanisms overlap with those of sunitinib, but its toxic effects can be milder than those of sunitinib (Motzer *et al.* 2013). In a phase 2 clinical trial with pazopanib (NCT1340794) (Jasim *et al.* 2017), the primary end point was ORR. Pazopanib was provided at a starting dose of 400 mg daily to test patients' tolerability. Two weeks later, the dose was increased to 800 mg daily. The trial included six evaluable patients. One patient achieved a PR that lasted for longer than 24 months. There were no CRs. Median PFS was 6.5 months. Substantial cardiovascular toxicity was noticed, especially when the dose of pazopanib was doubled. Half the patients had severe hypertension; two developed catecholamine-induced cardiomyopathy and had to discontinue trial participation. The trial was terminated due to low accrual.

Axitinib

Axitinib inhibits VEGFRs 1–3 and the PDGFR- β . Axitinib was approved for treatment of clear cell renal cell carcinoma at a starting dose of 5 mg twice daily; this dose is increased to 7 or 10 mg twice daily as long as the patient's blood pressure remains lower than 150/90 mmHg (Rini *et al.* 2013). The antineoplastic effects in patients with clear cell

renal cell carcinoma are more obvious with doses higher than 5 mg twice daily.

Axitinib is a more potent antiangiogenic medication than are pazopanib and sunitinib. In addition, axitinib does not inhibit c-Kit or FLT3 receptors and may be easier to tolerate. However, axitinib frequently causes severe hypertension (van Geel *et al.* 2012). A phase 2 clinical trial with axitinib for patients with progressive MPPGs was developed (NCT01967576) (Burotto Pichun *et al.* 2015). The primary end point was ORR. The trial recruited 14 patients. Preliminary results derived from 11 patients showed 4 patients with a PR (36%) and 6 with SD (55%), including two individuals with some degree of regression (16–18% reduction); only one patient did not respond to therapy. There were no CRs. Among study patients, 82% developed severe hypertension and none tolerated a dose of axitinib ≥ 5 mg twice daily. The trial is active but closed to recruitment.

Cabozantinib

Cabozantinib inhibits VEGFR-2, and the RET and c-MET receptors. The drug is approved for treatment of medullary thyroid, clear cell renal cell, and hepatocellular carcinomas. Several aspects support the evaluation of cabozantinib for patients with MPPGs. Cabozantinib is the most potent antiangiogenic drug available in clinical practice (Table 1). Inhibition of the c-MET pathway could be of interest in patients with MPPGs because activating somatic mutations of the c-MET receptor have been described in these tumors and the pathway activity is enhanced by pseudohypoxia through HIF-1 α stabilization; in addition, VHL protein inactivation increases the activation of C-met (Trusolino *et al.* 2010, Gherardi *et al.* 2012, Toledo 2017, Pozas *et al.* 2019).

Activation of the c-MET pathway contributes to cancer invasiveness, metastases, and escape from apoptosis. Furthermore, the c-MET pathway is upregulated in response to the blockage of the VEGF pathway, leading to the emergence of tumor resistance (Trusolino *et al.* 2010). Cabozantinib may prevent tumor resistance and metastases. In fact, cabozantinib has been associated with more impressive oncological and durable responses in patients with clear cell renal cell carcinoma than has sunitinib (Choueiri *et al.* 2017). Cabozantinib is also a more potent inhibitor of the RET receptor than sunitinib is and could be an effective treatment for the rare MEN2-MPPGs (Table 1).

Finally, cabozantinib improves clinical outcomes in patients with cancer and bone metastases (Smith *et al.* 2016);

thus, cabozantinib should be explored in patients with MPPGs and bone lesions. A phase 2 clinical trial with cabozantinib for patients with MPPGs is currently active (NCT02302833) (Jimenez *et al.* 2017). This trial allows participation of patients with bone metastases. The primary end point is ORR. Patients are treated with cabozantinib at 60 mg daily, and the dose is reduced to 40 or 20 mg daily depending on patients' tolerability. The clinical trial has recruited 17 patients. Preliminary results derived from 11 patients identified 4 patients with PR (37%) and 6 patients with SD (55%); all patients with SD had tumor regression (18–29%). The DCR was 92%. PFS was 16 months (range, 0.9–36 months). One patient did not respond to therapy. Responders included patients with apparently sporadic MPPGs as well as carriers of *SDHB* mutations. Unlike the previously described trials, patients treated with cabozantinib have not experienced severe hypertension or cardiovascular events and no patients have discontinued trial participation. The trial is currently recruiting patients.

Lenvatinib

Lenvatinib inhibits VEGFR 1–3, PDGFR- β , and FGFR1 (Zschäbitz & Gröllich 2018). The drug is approved for treatment of follicular origin thyroid, clear cell renal cell, and hepatocellular carcinomas (Hao & Wang 2020). Lenvatinib has been associated with impressive ORR in these tumors. However, hypertension has been reported as a common and frequently severe adverse event. A patient with a MPPG associated with a mutation of the *SDHB* gene who was treated with lenvatinib had a rapid and impressive radiographic response (Jasim *et al.* 2016). A phase 2 clinical trial with lenvatinib has been proposed (NCT03008369) with ORR as the primary end point. The trial is active and has recruited three patients, but recently stopped recruitment. No results have been reported.

Important aspects to consider when developing clinical trials with antiangiogenic drugs

Pharmacokinetics

Most antiangiogenics are oral medications with predominant liver metabolism through the cytochrome P450 3A4 (CYP3A4) enzyme (Table 2); thus, drug interactions are possible (www.drugs.com).

For instance, grapefruit consumption increases the levels of antiangiogenic drugs and must be avoided. The use of antiangiogenics along with diltiazem, amiodarone, and some beta-blockers must be reviewed with specialists in pharmacology and cardiology, since some interactions may increase the risk of cardiovascular toxicity. The concomitant use of bisphosphonates for bone metastases may increase the risk of osteonecrosis of the jaw. Strict dental hygiene is recommended.

Results of clinical trials with antiangiogenic therapy for MPPGs show that clinical responses, tolerability, and toxicity vary among patients. In addition to drug interactions and the presence of co-morbidity (i.e. liver failure), genetic aspects such as CYP3A4 polymorphisms may contribute to variability in response to anticancer drugs (Preissner *et al.* 2013). Currently, pharmacological researchers are looking for tests that can assess CYP3A activity to predict doses, improve clinical outcomes, and/or reduce the intensity and frequency of adverse events (Diekstra *et al.* 2014, García-Donas *et al.* 2016).

Cardiovascular toxicity

Clinical trials with antiangiogenic drugs for MPPG have encountered cardiovascular problems, patients have frequently discontinued trial participation, and trial success has been compromised (Jimenez 2018, Jasim & Jimenez 2019). Preliminary results from a phase 2 trial with cabozantinib have shown no severe cardiovascular events. Patients with MPPGs are very different from those with other cancers for which antiangiogenic drugs are approved, for several reasons: (1) MPPGs release catecholamines, whereas other types of tumors do not; (2) patients' tumor burden is frequently heavier than that in other malignancies; and (3) clinical experience with MPPGs is limited. Patients treated with antiangiogenic medications must be well prepared with antihypertensive medications and carefully monitored by clinicians with experience in catecholamine toxicity. Hormonal behavior is not predictable, and antihypertensives may need frequent and aggressive adjustments. In addition, the dose of the antiangiogenic drug must be carefully chosen. To date, the trial with cabozantinib suggests that small doses may bring oncological benefits with no major cardiovascular toxicity.

Tumor resistance

Preliminary results indicate that antiangiogenic medications do not cure MPPGs. However, the medications

offer antineoplastic effects and stop tumor progression for some time. Resistance at the outset of antiangiogenic therapy is rare (intrinsic resistance); in fact, results of clinical trials with axitinib, cabozantinib, and sunitinib describe an elevated DCR. The mechanisms of intrinsic resistance in the rare cases that do not respond are unknown. Acquired resistance happens in most patients and becomes apparent months to years after initiation of treatment. Mechanisms of acquired resistance include the following: (1) activation of proangiogenic signaling pathways that are not induced by inhibition of the VEGF pathway, (2) subsequent recruitment of endothelial progenitors from bone marrow that are resistant to current tyrosine kinase inhibitors and that release proangiogenic factors that build new vessels, (3) utilization of normal tissue vessels in close proximity to the tumor cells as a supply of oxygen and nutrients (vessel co-option), (4) transformation of tumor cells into endothelial-like cells (vascular mimicry), (5) vascular depletion due to high antiangiogenic drug concentrations that increase hypoxia, promoting tumor progression and spread, (6) activation of pathways that lead to tumor spread (c-met pathway), and (7) tumor symbiosis mediated by overactivation of mammalian target of rapamycin (mTOR) signaling (Bergers & Hanahan 2008, Zarrin *et al.* 2017). There are no reliable models of MPPGs, and the evaluation of these mechanisms of resistance is very difficult (Lussey-Lepoutre *et al.* 2018). However, final results from some of the phase 2 trials may provide some clues. For instance, the cabozantinib trial does not use maximal doses; this trial may favor the antiangiogenic therapeutic effect of vascular normalization instead of vascular depletion.

Therapeutic combinations

Antiangiogenic effects may favor other systemic therapies. HSA-I-131-MIBG was recently approved in the United States for the treatment of patients with MPPGs, and Lu-177-DOTATATE is currently being evaluated in clinical trials (NCT03206060 and NCT04106843). These drugs release lethal radioactivity to MPPGs. The vascular normalization caused by antiangiogenic medications may facilitate the radiopharmaceuticals approach to these tumors by enhancing the release of radioactivity. A recent report described a patient treated with a combination of sunitinib and I-131-MIBG who achieved CR for some time (Makis *et al.* 2016).

Immunotherapy is revolutionizing the oncology world. Immunotherapy has changed clinical outcomes in many patients. For instance, some patients with

melanoma are now cured with immunotherapy. However, immunotherapy does not work in every cancer. The combination of antiangiogenic drugs with immunotherapy is exciting (Motzer *et al.* 2019). Targeting the VEGF pathway enhances the efficacy of immunotherapy (Taylor *et al.* 2020). Vascular normalization activates adhesion molecules and chemokines that recruit and attract cytotoxic T cells; furthermore, vascular normalization facilitates immune cell mobilization. Many trials combining checkpoint inhibitors and antiangiogenic drugs to treat various malignancies are active; these trials aim to provide a tumor microenvironment that facilitates immune recognition and subsequent attack of cancer cells.

Cluster 1 tumors overexpress the mTOR pathway, and activation of mTOR signaling may lead to tumor symbiosis and resistance to antiangiogenic therapy (Allen *et al.* 2016, Oudijk *et al.* 2017). Everolimus, an mTOR inhibitor, was evaluated as single therapy in a clinical trial for patients with MPPGs; results from this trial indicated that everolimus alone was not an effective drug (Oh *et al.* 2012). However, combining antiangiogenic medications with mTOR inhibitors may lead to more effective and prolonged responses. This has been suggested by a case report of a patient with an MPPG treated with sunitinib and sirolimus (Ayala-Ramirez *et al.* 2012). A clinical trial is needed to evaluate this therapeutic combination.

Finally, PT2977 – a potent inhibitor of HIF- α – has demonstrated antineoplastic effects in clear cell renal cell carcinomas, including occasional CR (Xu *et al.* 2019). Observations in other malignancies, however, have shown that antiangiogenic therapies may at some point stimulate tumor invasion due to increased hypoxia, glycolysis, and induction of HIF- α , despite vascular normalization (metabolic adaptation) (Keunen *et al.* 2011, Fack *et al.* 2015). Combining PT2977 with antiangiogenic drugs may lead to more impressive responses. Currently, the combination of cabozantinib with PT2977 is being evaluated in a phase 2 clinical trial for kidney cancer (NCT03634540).

Conclusions

Systemic therapy for MPPGs includes antiangiogenic medications. These drugs have antineoplastic effects, including tumor size reduction and disease stabilization, but have been associated with cardiovascular toxicity,

especially in hormonally active tumors. The adverse effects from these medications, however, are in large part preventable and controllable. Antiangiogenic medications represent an alternative for patients with progressive disease and provide an opportunity to improve therapeutic interventions and clinical outcomes. To help ensure these outcomes, patients with MPPGs should be monitored and treated by a multidisciplinary team that is familiar with the oncological, hormonal, and pharmacologic aspects of this disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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