Review Article

c-Met expression in renal cell carcinoma with bone metastases

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ABSTRACT

Hepatocyte growth factor (HGF)/c-Met pathway is implicated in embryogenesis and organ development and differentiation. Germline or somatic mutations, chromosomal rearrangements, gene amplification, and transcriptional upregulation in *MET* or alterations in autocrine or paracrine c-Met signalling have been associated with cancer cell proliferation and survival, including in renal cell carcinoma (RCC), and associated with disease progression. HGF/c-Met pathway has been shown to be particularly relevant in tumors with bone metastases (BMs). However, the efficacy of targeting c-Met in bone metastatic disease, including in RCC, has not been proven. Therefore, further investigation is required focusing the particular role of HGF/c-Met pathway in bone microenvironment (BME) and how to effectively target this pathway in the context of bone metastatic disease.

1. Introduction

Bone metastases (BMs) are an important clinical issue in several tumor types, particularly prostate, breast, lung, and kidney, and are associated with severe comorbidities related with skeletal-related events (SREs), including severe bone pain, spinal cord compression, pathological fracture, and hypercalcemia [1]. Although subject of intensive research, several questions remain unanswered due to the complexity of bone microenvironment (BME), key for cancer cell survival [1,2].

Bone is one of the most common metastatic sites in renal cell carcinoma (RCC), together with lung, liver, lymph nodes, and brain [3]. In RCC, 20–35% of patients with advanced disease develop BMs, mostly osteolytic lesions [3,4], a negative prognostic factor associated with a 10.2-month OS decrease and severe morbidity [5].

Time from nephrectomy to BMs development is an important prognostic factor [6]. Patients with BMs should be identified as early as possible and treated accordingly. A comprehensive survey in 398 RCC patients with BMs risk-stratified patients according to the Memorial Sloan-Kettering Cancer Center (MSKCC) score [7] and reported a median time to BMs diagnosis of 24 months for good-risk patients, five months for intermediate-risk patients, and zero months for poor-risk patients [8]. In addition, 71% of patients experienced at least one SRE.

It should be acknowledged that not all RCC patients with BMs have poor survival. Some patients with oligometastatic disease can be long survivors, especially if submitted to surgical treatment [9–13]. Several studies have shown that radical resection of oligometastatic disease is an important prognostic factor, suggesting that bone surgery should be considered to achieve local tumor control and increase OS in this subset of patients [9–13].

Bone targeting agents (BTAs), including bisphosphonates (BPs) and denosumab, widely used as standard of care in bone metastatic disease

[14], can also have a role in RCC in this setting. Although effective in preventing or delaying SREs, they have failed to improve overall survival (OS) [15]. Like for other tumor types, pre-clinical data suggest that BPs can induce apoptosis of RCC cell lines *in vitro* [16]. In the clinical setting, two studies have shown an improvement in progression-free survival (PFS) and OS in RCC patients with BMs treated with zoledronic acid in combination with targeted therapy [17,18]. However, a large pooled analysis of clinical trials including 2749 RCC patients with BMs showed no PFS (5.1 versus 4.9 months; $p = 0.1785$) or OS (13.3 versus 13.1 months; $p = 0.3801$) improvement for patients who received BPs versus those who did not [19].

Denosumab, a monoclonal antibody against receptor activator of nuclear factor-κB ligand (RANKL), is also a standard-of-care BTA, preventing osteoclast differentiation and survival [20]. A combined analysis of three randomized phase III trials evaluating the efficacy and safety of denosumab versus zoledronic acid in patients with BMs including 155 patients with RCC, showed denosumab superiority in delaying time to first SRE by a median of 8.21 months and reducing the risk of first SRE by 17% (HR 0.83; $p < 0.001$) [21], but no specific subgroup analyses was reported for patients with RCC [22].

c-Met is abnormally expressed in different tumors and has a prominent role in urogenital cancer [23]. Overexpression of c-Met and its hepatocyte growth factor (HGF) ligand, together with excessive HGF/c-Met signalling pathway activation has been reported in both clear-cell and papillary RCC (pRCC) [23–25]. HGF/c-Met pathway is also important in bone physiology and has been implicated in development of BMs, particularly in prostate cancer [26]. Additionally, c-Met has been shown to be overexpressed in RCC with BMs [27]. Overall, this provides the scientific rationale for c-Met inhibition as a potential therapeutic strategy in RCC, including in bone metastatic disease.

The tyrosine kinase inhibitor (TKI) cabozantinib – a multi-kinase c-Met, vascular endothelial growth factor 2 (VEGFR2), and AXL receptor tyrosine kinase (AXL) inhibitor – has shown activity in prostate cancer cells *in vitro* and in RCC models *in vivo* [28,29]. Cabozantinib has been shown to inhibit osteoclastogenesis by eliciting BME changes, supporting the role of HGF/c-Met pathway in BMs development and progression [28]. In the clinical setting, it has also been shown to be beneficial in RCC patients with BMs [29–31].

This review summarizes the relevance of c-Met expression in the development of BMs in cancer, highlighting its role in RCC.

2. HGF/c-Met pathway in cancer

MET is a proto-oncogene encoding for c-Met, a membrane-spanning receptor tyrosine kinase (RTK) [32]. HGF, c-Met activating ligand, is secreted by mesenchymal stromal cells, such as fibroblasts. HGF binding to c-Met induces receptor dimerization and trans-phosphorylation of two catalytic tyrosine residues, Tyr1234 and Tyr1235 [33,34]. Phosphorylation events induce downstream signal transduction via mitogen-activated protein kinase (MAPK) cascade, phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) axis, signal transducer and activator of transcription proteins (STATs), and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) [34]. c-Met activation leads to cell proliferation, survival, and migration, thus representing an important mechanism in cancer development [34].

Accordingly, c-Met aberrant expression is observed in several tumor types, being implicated in tumor progression, metastases development, and resistance to anti-epidermal growth factor receptor (EGFR), RAS-RAF-MEK, mammalian target of rapamycin (mTOR), and vascular endothelial growth factor receptor (VEGFR) therapies [35].

Different molecular alterations account for c-Met pathological activation. *TRP-MET* chromosomal translocation has been identified in gastric carcinoma [34]. Hereditary pRCC is characterized by trisomy of chromosome 7, together with missense mutations in *MET* tyrosine kinase domain coding region, with similar mutations found in sporadic pRCC [36]. *MET* amplification, leading to c-Met overexpression and
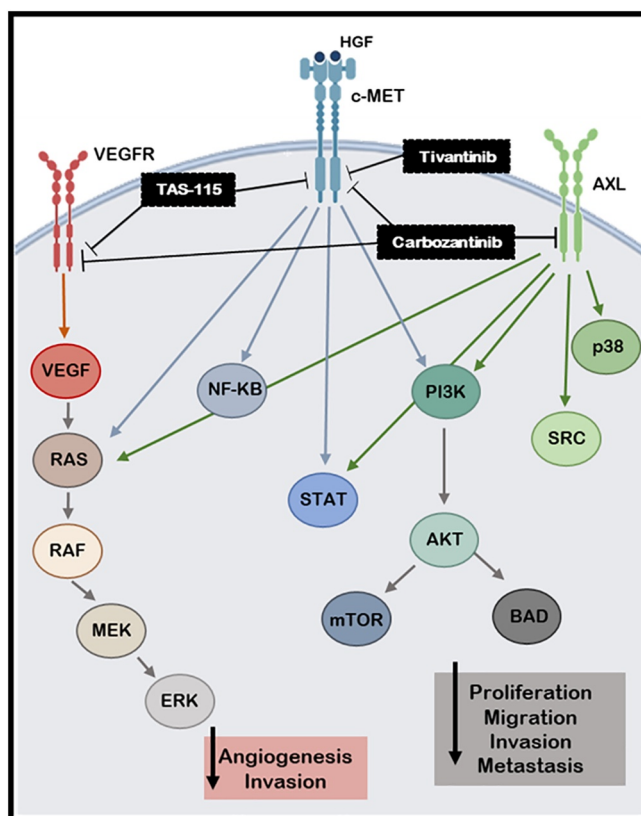

Fig. 1. Therapeutic targeting of HGF/c-Met pathway. c-Met pathway inhibition can be achieved using c-Met tyrosine kinase inhibitors, like cabozantinib, tivantinib, and TAS-115 (black boxes), through inhibition of several signalling pathways responsible for promoting proliferation, migration, invasion, and metastases formation. AKT serine/threonine-protein kinase, AXL AXL receptor tyrosine kinase, BAD BCL2 associated agonist of cell death, ERK extracellular signal-regulated kinase, MEK mitogen-activated protein kinase kinase , mTOR mammalian target of rapamycin, NF-κB nuclear factor kappa B, p38 p38 mitogen-activated protein kinase, PI3K phosphoinositide 3-kinase, RAS rat sarcoma virus homolog, RAF RAF serine/threonine-protein kinase, SRC proto-oncogene tyrosine-protein kinase, STAT signal transducer and activator of transcription, VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor.

constitutive activation, has been reported in non-small cell lung carcinoma (NSCLC), endometrial, gastro-esophageal and colorectal cancers, glioblastoma, and medulloblastoma [37]. However, c-Met constitutive activation driven by gene amplification is relatively rare, being more often attributed to *MET* overexpression induced by hypoxia and inflammatory cytokines or pro-angiogenic factors, such as interleukin (IL)-1α, IL-6 and tumor necrosis factor (TNF)-α, highly expressed in tumor microenvironment [37,38]. Finally, oncogene mutations, such as activated *Ras*, or oncosuppressors may also drive *MET* overexpression [39–41].

Several c-Met targeting therapies, including monoclonal antibodies (mAb) and TKIs, are currently in clinical development for use as single agents or in combination (Fig. 1). However, only cabozantinib and crizotinib have received approval for cancer treatment by the US Food and Drug Administration (FDA) and by the European Medicines Agency (EMA). Phase III trials targeting HGF/c-Met pathway are summarized in Table 1.

Multimodal treatment is also being investigated, through the combination of c-Met inhibitors with radiotherapy. Pre-clinical studies showed that c-Met is upregulated in irradiated cells, inducing treatment resistance [42–44], suggesting that c-Met inhibition could be useful in overcoming radiation resistance. Accordingly, c-Met inhibitors

Table 1
c-MET inhibitors in phase III trials of advanced-stage solid tumors.

Drug	Characteristics	Phase III trial	Cancer type
Rilotumumab	HGF neutralizing mAb	NCT01697072-RILOMET 1	Gastric and GEJ cancer
		NCT02137343-RILOMET-2	Gastric and GEJ cancer
		NCT02926638-LungMAP	SCLC
Onartuzumab	Fully humanized mAb binding to extracellular c-Met domain	NCT02031744	NSCLC
		NCT01887886	NSCLC
		NCT01662869	GEJ cancer
		NCT01456325-MetLung	NSCLC
		NCT02488330	Solid tumors
Crizotinib	Oral multi-target c-MET, ALK and ROS1 TKI	NCT01154140-PROFILE 1014	NSCLC
		NCT03126916	Neuroblastoma
		NCT03194893	ALK+ and RET+ cancer
		NCT02201992	NSCLC
		NCT04009317	NSCLC
		NCT02075840-ALEX	NSCLC
		NCT02838420	NSCLC
		NCT03052608	NSCLC
		NCT02767804-eXalt-3	NSCLC
		NCT02737501-ALTA-1L	NSCLC
Cabozantinib	Oral multi-target c-MET, VEGFR-1, -2, and -3, RET, AXL, KIT, TRKB, FLT-3, and TIE-2 TKI	NCT00704730-EXAM	Medullary thyroid cancer
		NCT03141177-CheckMate 9ER	RCC
		NCT03729245	RCC
		NCT03793166-PDIGREE trial	RCC
		NCT01522443-COMET-2 trial	CRPC
		NCT03375320	Neuroendocrine tumors
		NCT03937219-COSMIC 313	RCC
		NCT03755791-COSMIC 312	HCC
		NCT01865747-METEOR	RCC
		NCT01605227-COMET-1	CRPC
		NCT01908426-CELESTIAL	HCC
		NCT03690388	Thyroid cancer
Capmatinib	Potent oral, ATP-competitive, class I c-MET TKI	NCT03784014-MULTISARC	Soft tissue sarcoma
Savolitinib	Potent and selective oral c-MET TKI	NCT03091192-SAVOIR	Prcc
Tivantinib	Oral class III c-MET allosteric TKI	NCT02029157-JET-HCC	HCC
		NCT01377376-ATTENTION	NSCLC
		NCT01244191	NSCLC
		NCT01755767-METIV-HCC	HCC

ALK, anaplastic lymphoma kinase gene; ATP adenosine triphosphate; AXL tyrosine-protein kinase AXL; CRPC, castration-resistant prostate cancer; FLT-3, FMS-like tyrosine kinase 3; GEJ, gastroesophageal junction; HCC, hepatocellular carcinoma; HGF hepatocyte growth factor; KIT tyrosine-protein kinase KIT; mAb, monoclonal antibody; MET, MET proto-oncogene, receptor tyrosine kinase; NSCLC, non-small cell lung cancer; pRCC, papillary renal cell carcinoma; RCC, renal cell carcinoma; RET, rearranged-during-transfection proto-oncogene; ROS proto-oncogene tyrosine-protein kinase ROS; SCLC Squamous Cell Lung Cancer; TIE-2, Tyrosine-Protein Kinase Receptor TIE-2; TKI, tyrosine kinase inhibitor; TRKB Tropomyosin receptor kinase B; VEGFR vascular endothelial growth factor receptor.

enhanced radiosensitivity in different pre-clinical models [45–48].

HGF/c-Met pathway in RCC c-Met is expressed in tubular epithelial cells in the healthy adult kidney, where it stimulates cell growth [49]. c-Met is also important for branching tubulogenesis induction during tubule repair, following ischemic and chemical injuries or contralateral nephrectomy [49]. In contrast with the normal kidney, c-Met upregulation is usually observed in RCC, where it has been correlated with therapy resistance and disease progression, as recently reviewed by Marona P et al. [35].

The most common RCC subtype, clear-cell RCC (ccRCC), is extremely vascularized due to frequent loss-of-function mutations in the von Hippel-Lindau tumor suppressor gene (VHL), responsible for regulating HIF-1 (hypoxia-inducible factors) stability [24]. Loss of VHL activity results in HIF accumulation, leading to excessive VEGF or platelet-derived growth factor (PDGF) secretion and resulting in increased ability of tumor cells to metastasize [50,51]. HGF may also regulate VEGF expression and promote angiogenesis via c-Met activation [52]. Furthermore, it has been shown that VHL mutations together with a hypoxic environment lead to increased HGF and c-Met expression in ccRCC [53–55].

Given the relevance of angiogenesis and the VEGFR pathway in RCC, some anti-angiogenic molecules became standard-of-care in RCC treatment, such as sunitinib, pazopanib, and bevacizumab [56].

However, therapy resistance often occurs. It has been proposed that acquired resistance to anti-angiogenic therapies may occur as the result of epithelial-to-mesenchymal transition (EMT) and compensation of blocked receptors, as well as activation of alternative proteins or signalling pathways – such as HGF/c-Met pathway −, capable of driving tumor angiogenesis or growth independently of VEGFRs [57,58]. A study using xenograft mouse models showed that inhibition of AXL and MET activity may overcome induced resistance to sunitinib in metastatic RCC [59]. Based on this data, it has been proposed that a combination strategy targeting VEGF and HGF/c-Met could have significant survival impact and anti-tumor efficacy [60]. However, clinical studies are still required to corroborate this hypothesis.

A second hypothesis is that lack of endothelial cell influx caused by VEGF lack or blockade may cause vasculogenic mimicry within tumors, where blood vessel-like structures are formed by cancer cells [34].

Several studies have shown an association between c-Met expression and poor survival in RCC. A meta-analysis of 12 studies including 1724 patients with RCC showed that high c-Met expression was associated with high nuclear grade (2–4; odds ratio [OR] 2.45; 95% CI 1.43–4.19; $p = 0.001$) and high pT stage (pT3 and pT4; OR 2.18; 95% CI 1.27–3.72; $p = 0.005$) [61]. In addition, patients with c-Met-high RCC had decreased OS compared with patients with c-Met-low tumors (HR 1.32; 95% CI 1.12–1.56, $p = 0.0009$).

Table 2
Clinical trials of c-Met inhibitors in bone metastatic disease.

Study	Phase	Tested drugs	Eligibility	Results
COMET-1 NCT01605227 [72]	III	Cabozantinib vs prednisolone	mCRPC after docetaxel and enzalutamide/abiraterone	Median OS: 11 vs 9.8 months (HR 0.90; 95% CI 0.76–1.06; p = 0.213) Median PFS: 5.6 v 2.8 months (HR 0.48; 95% CI 0.40–0.57; stratified log-rank p < 0.001) Cabozantinib was associated with CTC conversion, bone biomarker normalization, and post-random assignment incidence of SSEs, but not with PSA outcomes.
COMET-2 trial NCT01522443 [73]	III	Cabozantinib vs mitoxantrone + prednisolone	Previously treated symptomatic mCRPC	Pain palliation at week 6, confirmed at week 12 (≥30% decrease from baseline in patient-reported average daily worst pain score via Brief Pain Inventory without increased narcotic use): 15% vs 17%, p = 0.8
METEOR trial NCT01865747 [29,30]	III	Cabozantinib vs everolimus	ccRCC after progression under anti-VEGFR therapy	Median PFS: 7.4 vs 3.9 months (HR 0.51, 95% CI 0.41–0.62, p < 0.0001) Median OS: 21.4 vs 16.5 months (HR 0.66, 95% CI 0.53–0.83, p = 0.00026) Sub-analysis of patients with BMs: Median PFS: 7.4 vs 2.7 months (HR 0.33, 95% CI 0.21–0.51) Median OS 20.1 vs 12.1 months (HR 0.54, 95% CI 0.34–0.84) SREs: 23% vs 29% Bone scan response per IRC: 20% vs 10% PFS, OS, and ORR were also improved with cabozantinib in patients without BMs. Increased normalization of bone resorption biomarkers in the cabozantinib arm.
JapicCTI-132333 [75]	I	TAS-115	Solid tumors refractory to standard treatment, with no available treatment options	Bone scan index (BSI) response rate in patients with bone lesions: 56.0%
NCT01575522 [77]	II	Tivantinib monotherapy	Metastatic triple negative breast cancer who have received prior 1 to 3 lines of chemotherapy in the metastatic setting	ORR: 5% (95% CI 0–25%) 6-month PFS: 5% (95% CI 0–25%)

BMs bone metastases; CI confidence interval; CRPC castration resistant prostate cancer; CTC circulating tumor cells; HR hazard ratio; IRC independent review committees; ORR overall response rate; OS overall survival; PFS progression free survival; RCC renal cell carcinoma; SSE symptomatic skeletal events

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These evidences support that c-Met can be an important target in RCC. The randomized phase III METEOR trial compared the efficacy and safety of the dual c-Met/VEGFR2 inhibitor cabozantinib with the mTOR inhibitor everolimus in patients with advanced RCC who progressed after previous anti-VEGFR therapy [29]. Compared with everolimus, cabozantinib significantly prolonged OS (median 21.4 vs. 16.5 months, HR 0.66; 95% CI 0.53–0.83; p = 0.00026) and PFS (median 7.4 vs. 3.9 months, HR 0.51; 95% CI 0.41–0.62; p < 0.0001). Based on these results, the FDA approved cabozantinib for treatment of advanced RCC patients who received prior anti-angiogenic therapy. It would be interesting to study if cabozantinib efficacy is HGF/c-Met specific or also depends on VEGF pathway inhibition.

Recent results of the phase III SAVOIR trial, comparing the c-Met inhibitor savolitinib with sunitinib in MET-driven pRCC, have shown an improvement in PFS (median 7.0 vs. 5.6 months, HR 0.71; p = 0.31), OS (median not reached vs 13.2 months, HR 0.51; p = 0.11], and ORR (27% vs 7%), although not statistically significant [62].

3. HGF/c-Met pathway in BMs

Bone marrow-derived CXCL12 is a major chemoattractant of CXCR4 and CXCR7-expressing cancer cells [63]. Following bone marrow homing, bone colonization and metastases onset rely on tumor interaction with BME, as recently reviewed by our group [2]. Tumor-bone interaction activates osteoclastogenesis and bone resorption, increasing release of tumor growth factors, such as bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), and fibroblast growth factor (FGF). Additionally, cancer cells secrete prostaglandins, parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP), activated vitamin D, IL-6, and TNF. These increase RANKL expression on osteoblasts and bone marrow stromal cells, further stimulating osteoclast proliferation, survival, and activity, eliciting osteolytic metastases [2,64]. BME immune compartment also regulates this process (specifically through T-cells), by blocking osteoclast activity and decreasing skeletal lesions and overall tumor burden [65]. Other cytokines, as IL-1, IL-6, and TGF-β, participate in this cycle by promoting the opposite effect: stimulating osteoclast growth and activation [64,66].

The relevance and expression of c-Met in bone was first described in a study showing that c-Met was expressed and activated by HGF in both osteoclasts and osteoblasts [67]. In osteoclasts, c-Met activation is followed by an increase in intracellular Ca^{2+} concentration and pp60c-Src kinase activation, eliciting alterations in osteoclast conformation, migration, and DNA replication. Osteoblasts respond to HGF by entering the cell cycle, as indicated by DNA synthesis stimulation [67].

Initial findings regarding c-Met role in the development of BMs in cancer came from studies on prostate cancer reporting c-Met overexpression in metastatic lesions, as well as an inverse correlation between c-Met expression and androgen receptor expression [26]. It was thus hypothesised that increased c-Met expression was related to disease progression and resistance to androgen deprivation therapy.

Subsequently, other studies, mainly in prostate cancer-induced BMs, demonstrated the role of the HGF/c-Met pathway in bone environment. In one study, cabozantinib showed a dose-dependent biphasic effect on osteoblast activity and an inhibitory effect on osteoclastogenesis *in vitro*, which reflected in prevention of prostate cancer-induced bone lesions *in vivo* [24]. This was due to c-Met blockade and VEGFR2 phosphorylation in prostate cancer cells and osteoblasts, respectively. Another study showed that cabozantinib inhibited subcutaneous prostate cancer cell growth in bone in a mouse xenograft model and tumor growth in a BMs mouse model [68]. The latter resulted in reduced bone response to tumor and increased bone volume. Cabozantinib has also been shown to have significant effects on BME, by reducing osteoclast and increasing osteoblast numbers compared to controls and eliciting changes in trabecular bone structure [69]. Finally, cabozantinib has been shown to reduce RANKL and macrophage colony-stimulating

factor (M-CSF) expression, with subsequent suppression of osteolysis and tumor growth [70].

These results supported clinical trials of cabozantinib in the treatment of patients with BMs.

Several HGF/c-Met inhibitors have been studied in clinical trials of c-Met inhibition in patients with BMs (Table 2).

In a phase II non-randomized expansion cohort study of metastatic castration-resistant prostate cancer (mCRPC), cabozantinib evidenced clinically meaningful pain palliation, reduced or eliminated patient narcotic use, and improved patient functioning [71].

Despite promising preliminary results, cabozantinib did not meet its pre-specified primary endpoint in phase III trials in mCRPC. In the phase III COMET-1 trial, cabozantinib displayed good bone scan response (42% vs. 3%, p < 0.001) and improved bone biomarkers, radiographic PFS, and circulating tumor cell (CTC) conversion, but failed to demonstrate an OS benefit [72]. In the COMET-2 trial, cabozantinib failed to improve pain palliation in patients with symptomatic BMs [73].

The c-Met/VEGF TKI TAS 115 showed activity in bone by supressing osteoclastogenesis and bone resorption in mouse xenograft models [70,74]. Results from a phase I trial showed a bone scan index decrease in 56% of patients, representing a decrease in the quantitative value of primary lesions or BMs [75].

In metastatic breast cancer, the c-Met inhibitor tivantinib suppressed BMs in an *in vivo* mouse model [76], but showed no PFS benefit in a phase II study [77].

The most common grade 3 or 4 adverse events reported with c-Met inhibitors are common to other TKIs and included diarrhea, fatigue, anemia, neutropenia, palmar-plantar erythrodysesthesia syndrome, and hypomagnesemia [29,72,73,76,77]. Multi-target agents, as cabozantinib, also targeting VEGFR, showed a high incidence of hypertension [29,72,73].

Overall, further research is required to sustain HGF/c-Met pathway targeting in bone metastatic disease and its association with metastatic pattern of bone lesions. Patients with bone-only metastases are often excluded from clinical trials, as it is very difficult to measure bone response and address bone lesions as the main target lesions in trials. RECIST 1.1 criteria consider BMs with soft tissue masses > 10 mm as measurable disease, which excludes most BMs [78]. In this setting, it would be useful to address BMs changes using other criteria, as the PERCIST criteria [79] or the MDA criteria [80].

Overall, despite evidence that HGF/c-Met pathway plays an important role in BME and BMs, further studies are required to fully understand its regulation, function, and role as therapeutic target.

4. HGF/c-Met pathway in RCC BMs

Various mechanisms and biomarkers have been studied in the context of BMs development in RCC, such as TGF-β, TGF-α/EFGR pathway, insulin mRNA binding protein-3 (IGF2BP3), cadherin-11, PTHrP, calcium/calcium-sensing receptor (CaSR), AKT/Integrin-α5 signalling, microRNAs, and HGF/c-Met [27,64,81–89]. However, it remains unclear why the incidence of BMs in RCC is so high.

As previously mentioned, c-Met/HGF pathway is implicated in RCC progression, and high c-Met expression in bone metastatic lesions is associated with poor prognosis, as reported in a retrospective analysis of nephrectomy and metastatic lesion specimens showing high c-Met expression in 86% of BMs [27]. It was therefore hypothesized that HGF/c-Met pathway could have a role in the development of BMs in RCC (Fig. 2). c-Met is very important in sustaining a mesenchymal, undifferentiated phenotype, and therefore key for the functional cancer stem cell (CSC) phenotype in some tumors [24,90]. CSCs have been shown to promote BMs formation in breast and lung cancer, suggesting that targeting these cells may prevent or block the metastatic process in bone [91,92]. More recently, it has been shown that the c-Met inhibitor JNJ-38877605 inhibited osteoclast activation and reduced osteotropic
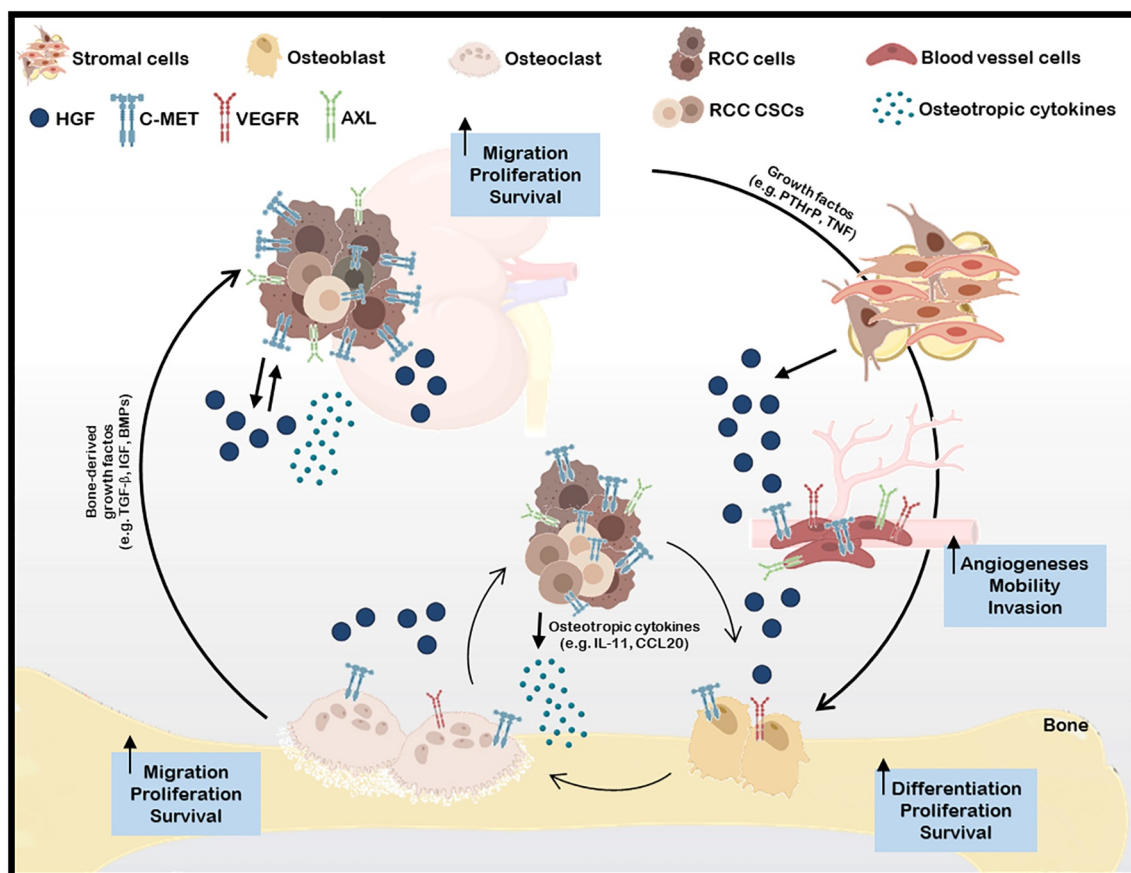
Fig. 2. Role of c-Met in RCC BMs. Tumor-bone interaction favors osteoclastogenesis and bone resorption, increasing release of growth factors that support tumor cell growth. c-Met is upregulated in tumor cells and in the bone microenvironment, being activated by hepatocyte growth factor (HGF) derived from stromal and tumor cells. Upon binding, HGF induces c-Met autophosphorylation, initiating downstream signalling cascades that promote proliferation, migration, invasion, and metastases formation. c-Met/HGF signalling on RCC stem cells (RCC CSCs) is important to sustain the undifferentiated phenotype of these cells and favours RCC cells metastization. The osteotropic cytokines IL-11 and CCL20 induced by RCC CSCs are increased in RCC patients with BMs and may also have an important role in the RCC metastatic process. AXL AXL receptor tyrosine kinase, BMPs bone morphogenetic proteins, IGF insulin-like growth factor, PTHrP parathyroid hormone-related peptide, RCC renal cell carcinoma, CSCs cancer stem cells, IL-11 interleukin 11, CCL20 chemokine (c-c motif) ligand 20, TGF- β transforming growth factor-β, TNF tumor necrosis factor, VEGFR vascular endothelial growth factor receptor.

cytokines IL-11 and CCL20 in a preclinical NOD/SCID mouse model, blocking BMs formation from c-Met-expressing RCC CSCs [93]. It was further shown that c-Met expression was increased in RCC patients with BMs and that systemic IL-11 and CCL20 were increased in these patients. Overall, these results suggest a relevant role for c-Met in RCC CSCs-induced BMs, but further studies are required to better understand the underlying mechanism.

Association of c-Met inhibition with survival and disease progression benefit in RCC patients with BMs has also been shown [30].

In a sub-analysis of the METEOR trial, comparing cabozantinib with everolimus after previous VEGFR therapy, a significant PFS (7.4 vs 2.7 months; HR 0.33; 95% CI 0.21–0.51) and OS (20.1 vs 12.1 months; HR 0.54; 95% CI 0.34–0.84) benefit was reported in the cabozantinib arm in patients with BMs (Table 2) [30]. In addition, also patients with both visceral and BMs benefited from the TKI compared with patients with bone-only metastases. Bone scan response (30% decrease in bone lesions) was observed in 20% of patients treated with cabozantinib compared with 10% of those treated with everolimus, and SRE incidence was 6% lower with cabozantinib. Additionally, a decrease in P1NP bone formation and CTx bone resorption biomarkers was observed in the cabozantinib arm. However, changes in bone biomarkers were observed in patients both with and without BMs and associated with cabozantinib pharmacodynamics [30]. These results suggest that c-Met inhibition has a beneficial effect in BME [30], with further studies currently ongoing to better understand the mechanism underlying c-

Met interaction with BME in RCC and how to better target it.

Following these results, the phase II RadiCaL clinical trial in RCC patients with BMs was designed to evaluate the efficacy of the combination of cabozantinib with radium-223, an alpha-emitting radioisotope and calcium mimetic that was shown to decrease SREs in patients with mCRPC and to prolong survival in patients with bone-only disease [94]. The combination of radium-223 with VEGF-targeting therapy had already been studied in mRCC in a phase I trial showing significant decline in bone turnover markers [95].

At present, there are no studies addressing the role of c-Met inhibitors in RCC, particularly in prevention of BMs, in the adjuvant setting. Three large clinical trials investigated whether there was a subset of patients that could benefit more from adjuvant treatment with these agents, showing contradictory results with VEGFR TKIs [96–98]. None reported specific subgroup analyses for patients with BMs. These trials used different prognostic models for patient stratification as intermediary/high-risk and none of them was prospectively validated [99]. In the CABOSUN phase II trial, cabozantinib showed significant PFS and ORR benefit compared with sunitinib in intermediate/poor-risk metastatic patients [31]. In subgroup analyses according to presence of BMs, cabozantinib also showed significant PFS improvement over sunitinib in patients with BMs (6.14 vs 3.38 months; HR 0.54; 95% CI 0.31–0.95), appearing to be an interesting alternative to sunitinib. As in pre-clinical studies cabozantinib showed an effect in BME regardless of presence of tumor cells [70], it would be interesting to investigate in

clinical studies in the adjuvant setting whether this translates in prevention of BMs through changes in bone remodelling in high-risk patients, more likely to develop metastases.

5. Conclusions

c-Met may be an important therapeutic target in RCC, as HGF/c-Met pathway is implicated in tumor cell survival and proliferation. Evidence also shows that the HGF/c-Met pathway induced by RCC CSCs has an important role in BME and may be implicated in BMs development; however, more studies are necessary. Although c-Met inhibition was shown to reduce skeletal lesions in the pre-clinical setting, clinical trials were not always successful in meeting their pre-specified primary endpoints. However, sub-analyses of phase III trials in RCC suggest an additional benefit of HGF/c-Met pathway blockage in patients with BMs, as well as a role in overcoming VEGFR TKI resistance. The impact of targeting this important bone metastatic disease pathway in other tumor types and how it changes their natural history is yet to be determined. Further research is also required to better understand the role of HGF/c-Met pathway in regulation of BMs microenvironment and how to better target it, not only through development of new drugs, but also by understanding drug resistance mechanisms and how to tackle them.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

The authors declare no conflicts of interest.

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