Tivantinib: a new promising mesenchymal–epithelial transition factor inhibitor in the treatment of hepatocellular carcinoma

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Tivantinib (ARQ 197) is an orally administered, selective small molecule that inhibits mesenchymal–epithelial transition factor (MET) via a novel, ATP-independent binding mechanism. Preclinical studies demonstrated that tivantinib has a broad-spectrum anti-tumor activity, especially in cells expressing high levels of MET. A randomized Phase II study in second-line hepatocellular carcinoma showed statistically significant improvement in time to progression with tivantinib compared to a placebo. Noteworthy, a significant pronounced benefit in time to progression and overall survival was observed in MET-high patients. In addition, MET expression was defined as a negative prognostic factor. The most frequent adverse events were hematologic events. A Phase III study in the MET-high hepatocellular carcinoma is actively recruiting patients. Phase II and III studies in non-small-cell lung cancer and colorectal cancer are ongoing.

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, representing the third leading cause of cancer-related deaths, with a rising incidence, particularly in Western countries [1]. Therapeutic approaches including hepatic resection, liver transplantation and locoregional therapies play a major role in the clinical management of HCC [2–4]. However, the high frequency of tumor recurrence and patients diagnosed at advanced stage requires systemic drug intervention. Current standard of care for patients with advanced HCC and a preserved liver function (Child-Pugh class A) is treatment with sorafenib, a small-molecule multitargeted receptor tyrosine kinase (TK) inhibitor (TKI), which blocks several receptor TKs involved in tumor cell proliferation and angiogenesis [5,6]. Other emerging targets for the treatment of advanced HCC include the mesenchymal–epithelial transition factor (MET) receptor TK and its sole ligand, HGF, which represent one of the most frequently deregulated signaling pathways in human cancer [7,8]. Stimulation of the HGF/MET signaling axis leads to the activation of multiple intracellular effectors, such as the Src/FAK, Ras/Raf/MEK/ERK and PI3K/Akt, which promote tumor cell growth, survival, migration and invasion, as well as tumor angiogenesis and metastasis. The aberrant activation of *MET* can occur through multiple mechanisms, including gene amplification,

transcriptional upregulation, point mutations, and HGF-mediated autocrine or paracrine activation [7,8]. Overexpression or enhanced activation of HGF or MET has been reported in a variety of human cancer types and correlates with increased metastases, cancer aggressiveness and poor prognosis [9]. Several studies demonstrated that the deregulation of the HGF/MET network is a common feature in HCC development and progression. MET overexpression is observed in 20–48% of human HCC samples and correlates with a significantly shorter 5-year survival rate [10–12]. A predictive model established according to *MET*-dependent gene expression signatures was able to define a subset of HCC characterized by an aggressive phenotype and poor prognosis [13]. In addition, the knockdown of *MET* by antisense RNA or RNAi strategies abrogated the *in vitro* and *in vivo* growth of HCC cells [14,15]. In this review, we discuss the preclinical and clinical data of tivantinib, an oral selective MET inhibitor, focusing the attention on HCC studies.

Overview of the market

Sorafenib was approved in 2008 for first-line systemic treatment of HCC, based on results of the SHARP trial, which reported a significant increase in survival and time to radiological progression over placebo [5]. The most frequent adverse events (AEs) were diarrhea,

Keywords

- hepatocellular carcinoma
- immunohistochemistry
- locally advanced/metastatic disease
- MET expression
- MET inhibitor
- second line
- tivantinib

hand-foot skin reaction, fatigue and weight loss, all of which were often manageable [5]. Although safety was not a major issue in the development of sorafenib, this might not be the case for other compounds where toxicities related to concomitant cirrhosis represent a concrete bottleneck, hampering their clinical development. In fact, despite promising data from two independent Phase II trials [16,17], a Phase III trial comparing sunitinib to sorafenib in patients with advanced HCC was discontinued due to concerns regarding safety profile, in addition to a lack of efficacy [18]. Furthermore, efficacy may also depend on the patient's characteristics and geographical origins. For instance, a parallel trial was concomitantly conducted to the SHARP trial [5] in the Asia-Pacific region [6], where HBV-related disease and more advanced stages are more prevalent compared with western countries. Results of this trial confirmed the magnitude of the benefit of sorafenib compared with placebo (hazard ratio [HR]: 0.68) [6].

In patients who are resistant, refractory or intolerant to sorafenib, no effective therapeutic options currently exist outside of clinical trials, and several targeted drugs are under evaluation in Phase II and III studies for second-line treatment. Most of these agents are designed to inhibit angiogenesis, an important process in HCC pathogenesis and progression, and include monoclonal antibodies (moAbs) or intracellular TKI. Brivanib, which targets both VEGF receptor (VEGFR) and FGF receptor, had the theoretical potential to overcome resistance to sorafenib. Nevertheless, a Phase III trial of brivanib versus placebo reported a median overall survival (OS) for the brivanib arm of 9.4 versus 8.2 months for the placebo arm (HR: 0.89; $p = 0.33$), thereby not meeting its primary end point [19]. Two other drugs are currently being tested against placebo in Phase III trials for regulatory approval. These include ramucirumab, a moAb against the VEGFR [101], and everolimus, an mTOR blocker approved for kidney cancer therapy [102]. Classifications of cancer subtypes based on molecular biomarkers have allowed for the identification of distinct oncogenic drivers, which may become both therapeutic targets and potential predictors of response. Three HCC subtypes were characterized by gene expression profiling [20], however, no molecular biomarker has been derived to predict response to targeted therapies and, specifically, to sorafenib. In a Phase II trial of sorafenib [21], the pretreatment levels of phosphorylated ERK were significantly correlated with time to progression

(TTP). Despite preclinical evidences of a correlation between phosphorylated ERK levels and sensitivity to sorafenib [22], these findings could not be further validated. Recently, in a correlative study of the SHARP trial [5], ten candidate plasma biomarkers were analyzed in both the placebo and the sorafenib arm. The angiogenesis biomarkers, Ang2 and VEGF, were reported to be independent prognostic markers of survival. Nevertheless, when authors tested the interaction between sorafenib treatment and each biomarker level at baseline, no biomarker was found to predict the benefit deriving from sorafenib over placebo [23,24].

The limited understanding of potential oncogenic drivers in HCC, partially related to the high molecular heterogeneity of the disease, has hampered the implementation of therapeutic strategies already pursued in other solid tumors. On the other hand, clinical experience with sorafenib indicates that simultaneous blockade of a wide kinase spectrum might represent an alternative approach to tackle, from a therapeutic standpoint, the heterogeneous molecular landscape of HCC.

Tivantinib

Tivantinib (formerly known as ARQ 197) is a highly selective, orally administered, non-ATP competitive inhibitor of the MET receptor TK, with an inhibitory constant (K_i) of 355 nmol/l in biochemical assays (ArQule, Inc. [103]; Daiichi Sankyo, Inc. [104]; Kiowa Hakko Kirin, Co., Ltd). In a large kinase panel screen, tivantinib has been shown to be 10–100-times more selective for MET than 229 other kinases tested. This agent presents a novel binding mechanism of action that facilitates the stabilization of an inactive nonphosphorylated configuration of MET, inhibiting autoactivation of the kinase.

Chemistry

Tivantinib (chemical formula (3R,4R)-3-(5,6-dihydro-4H-pyrrolo[3,2,1-ij]quinolin-1-yl)-4-(1H-indol-3-yl)pyrrolidine-2,5-dione) (FIGURE 1) is the most advanced representative of a newly discovered class of trans 3,4-disubstituted pyrrolidine-2,5-diones, which acts as a non-ATP competitive inhibitor of both constitutive and ligand-mediated MET phosphorylation [25].

Pharmacodynamics & preclinical studies Cancer cell line/*in vitro* studies

Tivantinib was discovered by a phenotype-driven process, using pathway-based assays in metastatic cancer cell lines. To assess the effect

of tivantinib on MET phosphorylation and its downstream effectors (Akt, ERK1/2 and STAT3), a wide range of human cancer cell lines (i.e., HT29, MKN-45, MDA-MB-231, and NCI-H441) were used [25]. Following a 72-h *in vitro* exposure to tivantinib, both constitutive and HGF-mediated MET activation were inhibited with an IC_{50} of 100–300 nmol/l. Moreover, the pharmacodynamic effects of tivantinib on cancer cells were fully comparable to those obtained through siRNA-mediated MET depletion. In a standard cytotoxicity assay, cancer cell lines expressing activated MET (i.e., MKN-45 and HT29) were strongly sensitive to tivantinib and showed caspase-dependent apoptosis, whereas cancer cells without detectable MET proteins (SK-MEL-28, NCI-H661, NCI-H446) were markedly less sensitive (ten-to-100-fold) [25]. These findings confirm the hypothesis that tivantinib exerts its antiproliferative and pro-apoptotic effects directly through the inhibition of MET and MET-dependent signaling pathway. *In vitro* assays revealed that the combination of tivantinib and sorafenib had synergistic or additive cytotoxicity against a large panel of human cancer cell lines (five of seven HCC lines tested) [26]; this effect was also noted for the combination of tivantinib and gemcitabine, especially with an intermittent schedule [27].

In vivo studies

To test the anti-tumor activity of tivantinib *in vivo*, three xenograft mouse models of human colon, pancreas and breast cancers (HT29, MKN-45, MDA-MB231) were evaluated. Following an oral administration of tivantinib at 200 mg/kg (serum concentrations higher than 300 nM for up to 12 h), significant tumor growth reductions of 45–79% ($p < 0.05$) were observed in all these xenograft models without significant body weight changes [25]. Pharmacodynamically, a single oral dose of 200 mg/kg of tivantinib induced a dramatic reduction in immunodetectable levels of phosphorylated MET in human colon xenograft tumors (HT29) 24 h after dosing. Consistent with *in vitro* data, tumor xenografts were exposed to sustained plasma levels of tivantinib (1.3 μ mol/l 10 h after dosing, value greater than threefold the inhibitory constant of tivantinib for MET) [25]. A dose-dependent antimetastatic activity of tivantinib was demonstrated in a humanized mouse model of metastatic breast cancer. At a daily dose of 120 mg/kg, tivantinib prevented the onset and progression of bone metastases and reduced the tumor-induced osteolysis [28]. Furthermore,

Figure 1. Tivantinib. Tivantinib is the most advanced representative of a newly discovered class of trans-3,4-disubstituted pyrrolidine-2,5-diones, which acts as a non-ATP-competitive inhibitor of both constitutive and ligand-mediated mesenchymal–epithelial transition factor phosphorylation.

the combination of tivantinib and sorafenib demonstrated synergistic activity in a xenograft model of non-small-cell lung cancer (NSCLC; NCI-H522), supporting the rationale for clinical development of this combination [26].

Preclinical pharmacokinetics

Tivantinib is rapidly metabolized by CYP2C19 (half-life = 2.8 min) and moderately metabolized by CYP3A4 (half-life = 16.3 min), without inhibiting any of the major CYP450 enzymes tested [29]. As shown by metabolic studies in animals and human hepatocytes, the oxidative biotransformation represents the primary metabolic pathway of tivantinib with an oral bioavailability $\geq 20\%$ [29].

Clinical pharmacokinetics

Clinical pharmacokinetic (PK) parameters of tivantinib were evaluated in three Phase I studies as monotherapy and in three combination studies with erlotinib, sorafenib and gemcitabine [27,30–34]. Tivantinib was administered at doses ranging from 10 to 400 mg twice daily (b.i.d.) and in three different active pharmaceutical ingredient forms (amorphous, crystalline A, and crystalline B). Crystalline B resulted in being the most stable and is currently being used in patients at 360 mg b.i.d. Overall PK profile of tivantinib was characterized by a moderate rate of absorption (peak 2–4 h after dosing), followed by a linear decrease in plasma concentrations approximately 4 h after dosing. Despite a wide interpatient variability, systemic tivantinib exposure generally increased over the dosing period. However, in most of cases, plasma levels of tivantinib grew less than proportionally with increasing dose. No relationships between drug-related

AEs, dose, baseline demographic variables and extent of tivantinib exposure were reported. CYP2C19*2 and CYP2C19*3 single nucleotide polymorphisms, relatively common in Asian subjects (35% allele frequency), resulted in enzyme deficiency and poor metabolism of tivantinib [31]. In combination with other agents, PK parameters of tivantinib appeared similar to those reported in monotherapy studies, indicating the absence of drug interactions [27,33,34].

Phase I trials

The initial safety and tolerability of tivantinib as a single agent has been explored in two Phase I, dose-escalation trials in adult patients with advanced solid tumors (TABLE 1).

In the first clinical evaluation in humans, two different dosing regimens of tivantinib were studied. After a favorable safety profile of an intermittent schedule (2 weeks on/1 week off) had been fully established, a continuous dosing regimen was introduced in a further 42 patients. Drug-related AEs of any grade were reported in 37 (46.8%) of the 79 patients enrolled [30]. Only two patients experienced dose-limiting toxicities (DLTs), including leukopenia, neutropenia, thrombocytopenia, vomiting and dehydration. Both of these patients received continuous dosing of tivantinib at 360 mg b.i.d. and the maximum tolerated dose was not reached because less than 33% of patients experienced DLTs at any given dose. The most common ($\geq 5\%$) drug-related AEs included fatigue (13.9%), nausea (13.9%), vomiting (10.1%), anemia (7.6%) and diarrhea (6.3%). The fatigue and gastrointestinal disorders were generally mild to moderate in severity and unrelated to dose, while an apparent dose-related increase in the frequency of anemia was reported, particularly at the 360 mg b.i.d. dose level. Among the 62 patients assessable for clinical activity, three patients (4.8%) achieved a partial response (PR), 38 patients (62%) showed stable disease (SD), whereas 20 patients (33%) progressed. Sixteen patients (25%) received tivantinib for more than 24 weeks and the median progression-free survival (PFS) was 12.1 weeks [30].

In order to identify the recommended Phase II dose (RP2D) using a continuous b.i.d. dosing schedule, a second Phase I study was conducted [30]. A total of 51 patients received tivantinib at doses ranging from 100 to 400 mg b.i.d. DLTs included grade 3 fatigue (200 mg b.i.d.; $n = 1$), grade 3 mucositis, palmar-plantar erythrodysesthesia, hypokalemia (400 mg b.i.d.; $n = 1$) and grade 3 febrile neutropenia (400 mg b.i.d.;

$n = 2$). All DLTs were resolved within 2 weeks of drug discontinuation. The maximum tolerated dose/RP2D of tivantinib was initially established as 300 mg b.i.d. (amorphous formulation), but was subsequently adjusted to 360 mg b.i.d. (PK equivalent) with the crystalline formulation [31]. Tivantinib was well tolerated with the most common toxicities being grade 1/2 fatigue (15.7%), nausea (13.7%) and vomiting (11.8%). Stable disease ≥ 4 months was achieved in 14 patients (27%), with minor tumor responses observed in gastric and Merkel cell carcinomas. Consistent with *in vitro* studies, pharmacodynamic analyses demonstrated that tivantinib administration decreased intratumoral phosphorylated and total MET levels, as well as phosphorylated FAK, with the concomitant induction of apoptosis as shown by terminal deoxynucleotidyl transferase dUTP nick end-labeling assays. Circulating endothelial cells declined significantly after treatment with tivantinib, while no significant changes in dynamic contrast-enhanced MRI parameters were observed [31].

In view of the growing evidence regarding the critical role of an aberrant HGF/MET network in HCC development and progression, a Phase Ib study was undertaken to evaluate the safety of tivantinib in 21 refractory patients with HCC and concomitant cirrhosis [32]. No drug-related worsening of liver function was observed and, therefore, tivantinib 360 mg b.i.d. was deemed the RP2D. Neutropenia was the most common drug-related AE and represented the primary reason for dose reduction, interruption or discontinuation. The frequency of any grade (52%) or severe (grade ≥ 3 ; 38%) neutropenia was markedly higher when compared with other dose-escalation trials, requiring a careful follow-up of hematologic toxicities in this population. Preliminary evidence of clinical activity in terms of SD was observed in nine of the 16 evaluable patients with a median duration of 4.6 months [32].

On the basis of strong preclinical evidences, dual EGFR-MET inhibition has been proposed as a strategy to overcome primary and secondary resistance to EGFR inhibitors in the clinical setting [35]. A total of 32 patients with metastatic solid tumors were treated with an escalating doses of tivantinib (120, 240 and 360 mg b.i.d.) in combination with a fixed dose of erlotinib (150 mg daily) [33]. Single or multiple intrasubject dose escalations were allowed in the absence of DLT through one cycle of therapy. The combination was well tolerated, with rash, nausea, abdominal pain, diarrhea,

Table 1. Phase I/II clinical trials of tivantinib.

Study (year)	Trial details	Outcome	Ref.
Phase I studies as a single agent			
Rosen <i>et al.</i> (2011)	Phase I dose-escalation study in 79 patients with advanced solid tumors. Two different dose schedules (intermittent and continuous) and 13 dose levels, ranging from 10 to 360 mg b.i.d. Three different active pharmaceutical ingredient forms (amorphous, crystalline A and crystalline B)	MTD not reached Most common drug-related AEs included fatigue (13.9%), nausea (13.9%), vomiting (10.1%), anemia (7.6%) and diarrhea (6.3%) Tivantinib 360 mg b.i.d. well tolerated, with mild-to-moderate toxicities	[30]
Yap <i>et al.</i> (2011)	Phase I dose-escalation study in 51 patients with advanced solid tumors; tivantinib doses ranging from 100 to 400 mg b.i.d.	Tivantinib MTD/RP2D established at 360 mg b.i.d. with the crystalline formulation	[31]
Santoro <i>et al.</i> (2013)	Phase Ib study in 21 patients with HCC and concomitant CP class A (n = 17) or B (n = 4) liver cirrhosis	No drug-related worsening of liver function Tivantinib 360 mg b.i.d. deemed the RP2D. Higher frequency of neutropenia	[32]
Phase I studies in combination with other agents			
Goldman <i>et al.</i> (2012)	Phase I dose-escalation study of tivantinib (120, 240 and 360 mg b.i.d.) in combination with a fixed dose of erlotinib (150 mg daily) in patients with advanced solid tumors	No formal MTD established. Tivantinib 360 mg b.i.d. in combination with erlotinib 150 mg considered as the RP2D. DCR 46.8%; 6/8 patients with NSCLC with long-lasting SD	[33]
Adjei <i>et al.</i> (2010)	Phase I dose-escalation study of tivantinib (240 and 360 mg b.i.d.) in combination with sorafenib (200 and 400 mg b.i.d.)	No formal MTD established. Tivantinib 360 mg b.i.d. in combination with sorafenib 400 mg b.i.d. considered as the RP2D. Preliminary evidence of anticancer activity in patients with RCC, HCC or melanoma	[34]
Martel <i>et al.</i> (2012)	Expansion phase in 20 HCC patients: 10 patients treated at the RP2D and 10 with tivantinib at 240 mg b.i.d.	ORR 10% (1 CR, 1 PR), DCR 75%. TTP for CP class A patients (n = 14) 7.3 months	[36]
Pant <i>et al.</i> (2010)	Phase Ib dose-escalation study of tivantinib (120, 240 and 360 mg b.i.d.) in combination with gemcitabine. Different dosing schedules: continuous, 3 weeks on/1 week off, 2 weeks on/1 week off, and 5 out of 7 days weekly	RP2D identified as tivantinib 360 mg b.i.d. continuously with gemcitabine 1000 mg/m ² weekly for 3 weeks on/1 week off. Following <i>in vitro</i> experiments, patients treated with a fixed gemcitabine schedule and pulsatile tivantinib. Promising anti-tumor activity in patients with breast, ovarian, uterine carcinoma and thoracic tumors	[27]
Phase II studies			
Santoro <i>et al.</i> (2012)	107 patients with locally advanced or metastatic HCC, failing first-line systemic therapy, randomized to tivantinib (71 patients) or placebo (36 patients); crossover allowed at radiographic disease progression (23 patients)	Increased TTP for the ITT population (6.9 vs 6.0 weeks). Most pronounced clinical benefit for MET-high patients, in terms of TTP (11.7 vs 6.1 weeks), PFS and OS (7.2 vs 3.8 months)	[41]
Sequist <i>et al.</i> (2011)	167 previously treated patients with NSCLC randomized to erlotinib with or without tivantinib	Prolonged adjusted PFS (adjusted HR: 0.61) for 117 patients with nonsquamous histology	[44]
Eng <i>et al.</i> (2012)	122 patients with <i>KRAS</i> wild-type pretreated mCRC treated with tivantinib plus irinotecan and cetuximab	In the Phase I trial portion ORR 44%; among three patients with MET-high tumors, 2 PR and 1 SD. Results of the Phase II trial are awaited	[50]

AE: Adverse event; b.i.d.: Twice daily; CP: Child–Pugh; CR: Complete response; DCR: Disease control rate; HCC: Hepatocellular carcinoma; HR: Hazard ratio; ITT: Intent-to-treat; mCRC: Metastatic colorectal cancer; MET: Mesenchymal–epithelial transition factor; MTD: Maximum tolerated dose; NSCLC: Non-small-cell lung cancer; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; PR: Partial response; RCC: Renal cell carcinoma; RP2D: Recommended Phase II dose; SD: Stable disease; TTP: Time to progression.

bradycardia and anemia being the most common drug-related AEs, mostly mild to moderate in severity. Two DLTs occurred at the 360 mg b.i.d. dose level. One patient developed grade 4 neutropenia on day 15, while a patient developed grade 4 neutropenia and leukopenia plus grade 3

thrombocytopenia on day 14. Two patients experienced arrhythmic serious AEs including sinus bradycardia and sick sinus syndrome. In the absence of a formally established maximum tolerated dose, given the favorable safety profile and preliminary signs of clinical activity, tivantinib

360 mg b.i.d. in combination with erlotinib 150 mg daily was considered the RP2D. One PR (3.1%) was seen in a microcystic adnexal carcinoma and SD occurred in 14 patients (43.8%). Six of the eight subjects with NSCLC achieved SD with a duration of 3–33 months. Of note, five out of six patients who had previously progressed to erlotinib monotherapy achieved SD with the combination therapy [33]. These encouraging results prompted further evaluation of this combination in NSCLC patients.

As reported above, the combination of tivantinib and sorafenib revealed synergistic and additive antiproliferative activity in preclinical studies [26]. To verify the safety and tolerability of this combination in the clinical setting, a Phase I dose-escalation study was performed in patients with advanced solid tumors [34]. Following the determination of the RP2D, an expansion cohort phase for patients with renal cell carcinoma, HCC, breast cancer, NSCLC and melanoma was opened. In the absence of DLTs, dosing was increased to the full single agent doses of both drugs (tivantinib 360 mg b.i.d. + sorafenib 400 mg b.i.d.) considered as the RP2D. A total of 62 patients were enrolled. The most common drug-related AEs of any grade were diarrhea (40%), rash (40%), fatigue (39%), anorexia (31%), hypophosphatemia (21%) and stomatitis (21%). A total of six serious AEs (all grade 3) were reported in four patients, including angina unstable, atrial fibrillation, diarrhea, febrile neutropenia, neutropenia and pneumonia. Preliminary evidence of anticancer activity was observed in patients with renal cell carcinoma, HCC and melanoma [36–38].

Among the 20 patients with HCC of the expansion phase, ten were treated at the RP2D and ten with tivantinib dose reduced at 240 mg b.i.d. after safety review of the HCC single-agent Phase II study. In this subgroup of patients, one complete response (CR), one PR and 12 SD were observed, accounting for an overall response rate (ORR) of 10% and a disease control rate of 75%. Interestingly, responses (one CR plus one PR) and stabilizations (three SD) were reported in eight HCC patients previously treated with sorafenib, suggesting that the combined inhibition of MET and angiogenic signals may have therapeutic potential in this setting. Of note, the patient who experienced CR presented a high MET expression [36].

The combination of tivantinib and gemcitabine was tested in a multicenter Phase Ib trial. A total of 65 patients with advanced solid tumors received tivantinib at escalating doses (120 mg

b.i.d., 240 mg b.i.d. and 360 mg b.i.d.) with different dosing schedules (continuous, 3 weeks on/1 week off, 2 weeks on/1 week off, and 5 of 7 days weekly) [27]. During the dose-escalation, drug-related AEs were reported in 57 patients (87.7%) with thrombocytopenia, anemia, neutropenia, fatigue and nausea being the most common toxicities. No DLTs were observed and the RP2D was identified as tivantinib 360 mg b.i.d. continuously with gemcitabine 1000 mg/m² weekly for 3 weeks on/1 week off. After encouraging clinical responses with the interrupted schedule, *in vitro* experiments demonstrated an advantage in growth inhibition for alternating or sequential schedules. In view of these results, the protocol was amended to treat patients with a fixed gemcitabine schedule (days 8, 15 and 22) and intermittent tivantinib (days 2–6, 9–13, 16–20 and 23–27) [27]. Promising anti-tumor activity was observed in patients with breast, ovarian, uterine carcinoma and thoracic tumors, including patients who progressed after prior gemcitabine therapy [39,40]. Expanded cohorts in these gemcitabine-sensitive tumor types are currently enrolling patients.

Clinical efficacy

Phase II study in HCC

The results of a multicentric, international, randomized, double-blind, placebo-controlled Phase II study of tivantinib in second-line unresectable HCC were recently published (TABLE 1) [41]. A total of 107 patients with cito-histologically confirmed locally advanced or metastatic HCC, progression or intolerance to first-line systemic therapy (103 sorafenib, four sunitinib), Child-Pugh class A cirrhosis, Eastern Cooperative Oncology Group performance status 0–1 and no prior liver transplant (stratified by performance status and presence/absence of vascular invasion) were randomized 2:1 to tivantinib or placebo, 71 patients on tivantinib and 36 on placebo. At radiographic progression, 23 patients on placebo crossed over to open-label tivantinib.

The primary end point was TTP in the intent-to-treat population, assessed by a central independent radiologist according to the Response Evaluation Criteria in Solid Tumors (version 1.1) [42]. Tumor measurements were performed every 6 weeks. Secondary end points included PFS, OS, ORR, disease control rate, crossover ORR, safety and pharmacokinetics. Furthermore, subgroup analyses were preplanned to evaluate efficacy according to MET status, viral status (HBV and/or HCV) and duration of prior systemic therapy.

Patients were enrolled from October 2009 to August 2011 in Europe and North America. After 57 patients were enrolled (38 in the tivantinib arm), a higher-than-expected incidence of grade >3 neutropenia was observed. As a consequence, the tivantinib dose of 360 mg b.i.d. was reduced to 240 mg b.i.d. in all patients and a modified dose reduction schema was implemented. Patients' characteristics were well-balanced among the study arms.

The study met its primary end point of TTP for the intent-to-treat population. Treatment with tivantinib produced a statistically significant 56% improvement in TTP, with a median of 6.9 weeks compared with 6.0 weeks (HR: 0.64; 90% CI: 0.43–0.94; $p = 0.04$). PFS was consistent with TTP with a HR of 0.67 (95% CI: 0.44–1.04) and a p -value of 0.06. Results in OS were not statistically significant. Of note, efficacy was comparable at 240 and 360 mg b.i.d., and at 240 mg b.i.d. fewer patients came off therapy or died due to side effects.

MET expression was evaluated by immunohistochemistry (IHC) assay using the anti-total MET (SP44) rabbit monoclonal antibody developed by Ventana Medical Systems (AZ, USA) at an independent central laboratory, after randomization and prior to unblinding. MET-high was defined as >50% of tumor cells with moderate or strong (2+ or 3+) staining intensity. A total of 30 patients (22 in the tivantinib and eight in the placebo arms) were not evaluable for MET expression due to tissue sample inadequacy, 45% of patients on tivantinib and 54% of patients on placebo were classified as MET-high. No significant imbalances were found in patients' characteristics when grouped by MET status.

On placebo, median OS was 3.8 months in MET-high patients compared with 9.0 months in MET-low patients (HR: 2.94; 95% CI: 1.16–7.43; $p = 0.02$), demonstrating the prognostic effect of MET.

The most pronounced clinical benefits in this study were observed in MET-high patients, the analysis of which was prespecified in the statistical analysis plan. These patients experienced a 133% improvement in TTP, with a median of 11.7 weeks compared with 6.1 weeks (HR: 0.43; 95% CI: 0.19–0.97; $p = 0.03$). PFS was consistent with TTP analysis, with a HR of 0.45 (95% CI: 0.21–0.95) and a p -value of 0.02. Disease control rate was 50% (range from 28 to 72%) on tivantinib versus 20% (range from 4 to 48%) on placebo. Median OS was 7.2 months on tivantinib versus 3.8 months on placebo (HR: 0.38; 95% CI: 0.18–0.81;

$p = 0.01$), representing a 163% improvement in OS. Of note, eight of the 15 placebo patients crossed over to open-label tivantinib and five remained on therapy for at least 6 weeks. In the MET-low population, efficacy results were statistically similar between the tivantinib and placebo arms.

Based on these results, an international Phase III study in second-line MET-high HCC is actively recruiting patients, to provide a new therapeutic option for this prevalent worldwide cancer.

Safety & tolerability

In the Phase II HCC study, the most commonly reported AEs (all grade) were asthenia, neutropenia, decreased appetite and anemia (TABLE 2). Among patients treated with tivantinib, the most frequent grade 3–5 AEs were neutropenia, observed in 21% of the patients at the dose of 360 mg b.i.d. but in only 6% of the patients at the dose of 240 mg b.i.d., and anemia, observed in 16% of the patients at the dose of 360 mg b.i.d. and 9% of the patients at the dose of 240 mg b.i.d. Five patients (four at the dose of 360 mg b.i.d.) with neutropenia also developed sepsis. Less frequent AEs included thrombocytopenia and hepatic failures, the latter of which were considered non-drug-related. Overall, AEs were similar in tivantinib and placebo arms, except for a higher incidence of hematologic events in the tivantinib arm and a manageable safety profile was observed with the dose of 240 mg b.i.d.

Other clinical indications

MET receptor activation is associated with poor prognosis and EGFR TKI resistance in NSCLC [43,35]. In a Phase II international randomized trial, 167 previously treated patients with EGFR TKI-naïve NSCLC were randomized to receive erlotinib (150 mg daily) with or without tivantinib (360 mg b.i.d.) (TABLE 1) [44]. PFS analysis, the primary end point of the study, favored the tivantinib arm, particularly in the preplanned adjusted analysis on the 117 patients with nonsquamous histology (HR: 0.61; 95% CI: 0.39–0.98; $p = 0.04$). A cohort of 15 patients with *KRAS* mutations achieved an even more significant benefit in PFS (HR: 0.18; 95% CI: 0.05–0.70; $p < 0.01$; interaction $p = 0.006$). Interestingly, patients on the tivantinib arm had a longer time-to-new metastatic lesions (HR: 0.49; 95% CI: 0.31–0.78; $p = 0.01$). Importantly, AEs were similar in both study arms and no unexpected toxicity was observed.

Table 2. Adverse events in the second-line randomized Phase II hepatocellular carcinoma study.

Preferred term	Patients (%)					
	Tivantinib 240 mg b.i.d. (n = 33)		Tivantinib 360 mg b.i.d. (n = 38)		Placebo (n = 36)	
	All grades	Grade 3–5	All grades	Grade 3–5	All grades	Grade 3–5
All AEs	100	49	95	55	86	44
Most common (>10%) AEs						
Asthenia	42	6	13	3	19	3
Neutropenia [†]	21	6	29	21	6	0
Decreased appetite	27	0	24	3	17	6
Anemia	21	9	21	16	6	0
Fatigue	12	3	29	8	28	6
Peripheral edema	24	6	18	0	17	0
Ascites	30	6	8	0	14	0
Diarrhea	9	0	24	0	25	3
Abdominal pain	9	3	16	0	19	3
Cough	12	0	13	0	14	3
Pyrexia	9	0	13	0	14	0
Other AEs of particular relevance						
Thrombocytopenia	9	6	11	5	0	0
Hepatic failure	6	6	5	5	0	0
SAEs						
AEs resulting in death	21		16		28	
All SAEs (all grades)	39		29		39	

[†]Includes five patients (four at 360 mg b.i.d.) who developed sepsis.
 AE: Adverse event; b.i.d.: Twice daily; SAE: Serious adverse event.

Consistent with the literature, at a retrospective, central IHC analysis in archival tissue samples, MET expression rate was found to be higher in nonsquamous (76%) than in squamous (12%) NSCLC [45,46]. The 25 nonsquamous, MET-high patients on tivantinib plus erlotinib showed an improvement in both PFS (HR: 0.58; $p = 0.28$) and OS (HR: 0.46; $p = 0.21$) compared with patients on erlotinib alone, while no detrimental effects were observed in patients with MET-low, nonsquamous tumors.

Results are expected from two randomized Phase III trials on nonsquamous NSCLC patients in western countries and Asia [105,106], while a Phase II study in patients with *KRAS* mutations is currently enrolling [107].

Finally, results are awaited for a Phase I/II study with tivantinib, irinotecan and cetuximab in *KRAS* wild-type, pretreated metastatic colorectal cancer patients (TABLE 1) [47]. The Phase I trial portion determined tivantinib 360 mg b.i.d. as the RP2D at full cetuximab dose, with ORR of 44%, and among three MET-high patients,

two partial responses and one stable disease have been observed. Interestingly, resistance to cetuximab has been associated to the activation of MET pathway in metastatic colorectal cancer [48].

Conclusion

Based on the above-mentioned studies, the anti-tumor activity of tivantinib is established in HCC as single agent and in combination with sorafenib. The main toxicities are asthenia, neutropenia, decreased appetite and anemia. The most pronounced clinical benefit was observed in MET-high HCC patients. As per our experience, the results of the Phase II trial in HCC represents the first randomized data reported with a MET inhibitor administered as a single agent in HCC, and the first identification of a biological subgroup of patients responding to a targeted therapy in HCC. Furthermore, MET expression was defined as a negative prognostic factor in second-line HCC. The Phase II results should be confirmed in a Phase III trial.

Future perspective

Predicting response to tivantinib:

IHC & FISH

A crucial step in the development of molecularly targeted therapeutics lies in the identification of those patients most likely to benefit from treatment. Although some preclinical studies have shown that in cancer cells with activated HGF/MET autocrine loop or *MET* amplification, treatment with a MET TKI impairs the tumorigenic and metastatic potential [49]. Other data on MET inhibition appear somehow conflicting as they result from cell lines in which mechanisms of MET constitutive phosphorylation remain poorly understood [50]. Furthermore, the concept that sensitivity to MET inhibitors may have a genetic basis is challenged by the hypothesis of *MET* as an ‘oncogene expedient’, which amplifies the effect of other oncogenes even in the absence of specific genetic alterations. Assays to quantify HGF/MET expression and activity have not been extensively validated. Two different assays have been tested in order to identify which patients most benefit from tivantinib and other MET inhibitors: IHC and FISH. In addition to the aforementioned HCC and NSCLC Phase II trials [41,44], the Ventana anti-total MET immunohistochemical assay is currently adopted in an ongoing Phase III study of erlotinib in combination with an anti-MET moAb (onartuzumab) to select patients with MET diagnostic-positive NSCLC [108]. Recent data suggest that levels of MET expression might also predict a benefit from chemotherapy combined with an anti-HGF moAb (rilutumumab) in advanced gastroesophageal cancers [51]. Among 90 patients, 42% had a MET diagnostic-positive status: in this subgroup of patients, rilutumumab added to chemotherapy conferred clinically relevant increases of PFS and OS over placebo [51].

Cut-offs being adopted to define MET overexpression and antibodies used may vary across different series and cancer types, so that an unequivocal definition for MET overexpression is still lacking. While IHC provides relevant spatial and morphological information, tissue fixation and paraffin-embedding might hamper antibodies validation [52].

MET amplification on chromosome 7 may cause protein overexpression and a constitutive activation of the TK domain. *MET* copies as a predictor of response were retrospectively investigated by FISH in a Phase II randomized trial of tivantinib plus erlotinib versus placebo in advanced NSCLC [44]. Patients

with four or more *MET* copies demonstrated a trend toward increased PFS under erlotinib plus tivantinib treatment. Benefit grew in magnitude as gene copy number increased, but statistical significance was not reached. Interestingly, *MET*:CEP7 ratios greater than two (which characteristically discriminate chromosome polysomy from true gene amplification) were observed in only three patients (2%) [44]. Study outcomes were further explored according to MET protein expression by IHC [45]. However, in the intent-to-treat population, the benefit derived from erlotinib plus tivantinib over erlotinib plus placebo was observed in both MET-high and MET-low subgroups of patients [45]. Numerical abnormalities of chromosome 7 have been reported in HCC. *MET* amplification by FISH was reported in one of 44 HCC patients, whereas chromosome 7 polysomy was detected in an additional 17 patients [53]. It is unknown whether *MET* copy number might predict sensitivity to tivantinib in HCC. Nevertheless, these data seem to rule out the possibility that true *MET* amplification may play a role in predicting outcome to tivantinib in both NSCLC and HCC. By contrast, the correlation between chromosome 7 polysomy and outcome appears to be more sound and warrants further investigations.

Other MET-targeted therapies under development in advanced HCC

Currently, multiple anti-MET compounds are under study in HCC and in several tumor types, some of which are now entering Phase II and III trials. These targeted therapies include HGF and MET moAbs, which typically interfere with ligand and receptor interactions, and small-molecule TKIs directed to target receptor activation. Two additional classes of agents, in their early development stages, include decoys and antagonists that compete with ligand or receptor, and downstream inhibitors of MET.

Small-molecule TKIs can be further differentiated into selective TKIs, which specifically target MET in an ATP-competitive or noncompetitive manner, and unselective TKIs, which target MET as well as other kinases. Among selective MET TKIs being developed for advanced HCC, tivantinib has just completed the randomized Phase II trial stage. To our knowledge, no other selective MET TKI is being tested in similar development stages for second-line treatment of advanced HCC. Other highly selective MET TKIs, including EMD-1214063, LY-2801653, AMG-337, AMG 208 and INC-280, are rapidly

entering Phase I studies. JNJ-38877605, a small-molecule, ATP-competitive inhibitor of the catalytic activity of MET showed a 600-fold selectivity for MET in a panel of 250 tyrosine and serine-threonine kinases, and potently inhibited HGF-stimulated and constitutively activated MET phosphorylation [54]. A Phase I study with JNJ-38877605 was recently discontinued because of renal toxicities. Studies with the multi-target VEGFR and MET inhibitor XL184 (also known as cabozantinib) have shown significant activity against a number of solid tumors, including breast cancer, NSCLC, melanoma and castration-resistant prostate cancer. A Phase II discontinuation trial with XL184 in HCC patients has been recently presented [55]. In this study, where nearly half of patients were treatment-naïve for advanced disease, the PFS results suggest a potential activity of this compound, independent from previous treatment. Interestingly, 38% of patients achieved a decline of AFP levels, which was demonstrated to

correspond to an increase in survival in patients treated with sorafenib [56].

Other unselective MET TKIs include foretinib (XL880), which is an orally available compound that mainly inhibits MET and VEGFR2 [57]. The results of a recently published study show that molecular targeting of AXL, VEGFR2 and MET by foretinib efficiently inhibits the growth of HCC xenografts and significantly prolonged mouse survival [58]. A Phase I/II trial is ongoing in patients with advanced HCC [109].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Mechanism of action

- Tivantinib is a highly selective, orally administered, non-ATP-competitive mesenchymal–epithelial transition factor (MET) inhibitor, which strongly inhibits MET autoactivation by selectively targeting the inactive nonphosphorylated form of the kinase.
- Tivantinib arrests MET-dependent downstream signaling by disrupting constitutive and ligand-mediated MET phosphorylation.

Pharmacokinetic properties

- After oral administration, the pharmacokinetic profile of tivantinib is characterized by a moderate rate of absorption (peak 2–4 h after dosing), followed by a linear decrease in plasma concentrations approximately 4 h after dosing.
- A considerable interpatient variability in the pharmacokinetics of tivantinib was observed.
- Systemic tivantinib exposure generally grows with increasing doses, often less than proportionally.
- No relationships between drug-related adverse events, dose, baseline demographic variables and extent of tivantinib exposure were reported.
- Tivantinib is rapidly metabolized by CYP2C19 and moderately metabolized by CYP3A4, without inhibiting any of the major CYP450 enzymes.

Clinical efficacy

- In second-line hepatocellular carcinoma (HCC), tivantinib significantly increased time to progression compared with placebo.
- The most pronounced clinical benefits were observed in second-line MET-high HCC: tivantinib significantly increased time to progression and overall survival compared with placebo.
- In non-small-cell lung carcinomas prolonged progression-free survival compared with placebo was observed with tivantinib; the most pronounced benefit was observed in nonsquamous tumors.

Safety & tolerability

- The main adverse events are asthenia, neutropenia, decreased appetite and anemia.
- In HCC, no significant increase of adverse events compared with placebo was observed, except for a higher incidence of hematologic events.
- In HCC, a manageable safety profile was observed with the dose of 240 mg twice daily.

Drug interactions

- Coadministration of tivantinib with other anticancer agents does not alter the pharmacokinetic parameters of tivantinib, indicating an absence of drug–drug interactions.

Dosage & administration

- In HCC, a regimen of tivantinib 240 mg twice daily was identified by the Phase II trial as the most appropriate.
- In other solid tumors, a regimen of tivantinib 360 mg twice daily, as monotherapy or in combination, was identified by Phase I trials as the most appropriate.

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