Apatinib: A novel receptor tyrosine kinase inhibitor for the treatment of gastric cancer

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ABSTRACT

Metastatic gastric cancer is a lethal disease characterized by a very short overall survival, underlining a critical need of new therapeutic options. Unfortunately, although several molecular targets have been investigated, only very few recently approved agents, such as trastuzumab in the HER2-positive setting and ramucirumab, led to a clinical improvement in the outcome of metastatic gastric cancer patients. VEGF (vascular endothelial growth factor) is one of the most potent angiogenic factors and is a signaling molecule secreted by many solid tumours. Since high VEGF expression is one of the characteristic features of gastric carcinomas, targeting VEGF is therefore considered as a promising therapeutic strategy for gastric cancer. In the scenario of possible new target therapies with particular regard to angiogenesis, apatinib is a novel receptor tyrosine kinase inhibitor selectively targeting VEGFR-2. It is an orally-bioavailable agent currently being studied in several solid tumour types showing a promising activity in gastric cancer. Due to the recent positive results as a third line of treatment for metastatic gastric cancer patients, apatinib may be an interesting and novel type of targeted treatment for metastatic gastric cancer in several lines of therapy. In this review, we summarize the available data of apatinib, mainly focused on the clinical aspect, in advanced/metastatic gastric cancer.

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Introduction

Gastric cancer (GC) is one of the most common malignancies and has been estimated to account for the third cause of cancer deaths in year 2015 [1]. Although different drugs are currently available for GC, the prognosis for the metastatic setting still remains poor [2,3]. During the last years, conventional chemotherapeutic agents reported 5-year survival values as low as 10% and an overall survival (OS) limited to 1 year in the metastatic setting [4]. However, more recently some innovative and biological therapies have become reality for the treatment of GC patients. The TOGA trial has been the first randomized phase III study, which demonstrated an advantage in terms of progression free survival (PFS) and OS for patients with positive human epidermal receptor 2 (HER-2) GC tumours [5]. Unfortunately, only a small percentage of patients (approximately 20%) are ideal candidates for HER-2 targeted therapy [5–7]. Additionally, ramucirumab, a monoclonal antibody against vascular endothelial growth factor receptor (VEGFR)-2, has shown a survival benefit as a second line treatment option in those metastatic GC patients who progressed on fluoropyrimidine- or platinum-based first-line chemotherapy [8,9]. On the other hand, other antiangiogenic agents such as bevacizumab, sunitinib and sorafenib failed to demonstrate a survival advantage [10]. However, recent studies support the inhibition of angiogenesis as an area of considerable interest with regard to GC research [11]. In this contest, apatinib, a novel receptor tyrosine kinase inhibitor selectively targeting VEGFR-2 [12,13], generated positive results in first preclinical and clinical studies involving GC patients. Apatinib mesylate is a new inhibitor of VEGF-2 tyrosine kinase targeting the intracellular ATP-binding site of the receptor. The aim of this mini-review is to summarize the first preliminary available data on apatinib in advanced/metastatic GC. Finally, future directions will be discussed.
The molecular basis of tumour angiogenesis

In physiological conditions, angiogenesis is a process responsible for the generation of new blood vessels in order to provide oxygen and nutrients to peripheral tissues and to maintain correct levels of perfusion. However, when the same process becomes uncontrolled such as in the case of cancer, the formation of new blood vessels becomes a pathological factor, which dramatically worsens patients’ clinical outcomes. Angiogenesis is today universally considered as a cancer hallmark, as it supplies for the increased request of oxygen and nutrients which is typical of the fast-growing microenvironment of solid tumours; as a result, tumour angiogenesis provides for the formation of new blood vessels in an un-controlled and not-organized fashion, ultimately resulting in enhanced tumour growth and increased metastatic potential.

The angiogenic process is majorly regulated by the interactions between tissue VEGFRs and their soluble ligands (VEGFs), which are kept under a finely regulated control in healthy conditions. VEGF molecules consist of several sub-variants, including VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF). Similarly, the receptor family involves three molecular subtypes (VEGFR-1, VEGFR-2 and VEGFR-3) which are type-II transmembrane proteins characterized by a tyrosine kinase (TK) activity. Among these receptors, VEGFR-2 has been found to be the one which is majorly implicated in the pathological over-formation of blood vessels in the context of several solid tumours (Fig. 1).

VEGFR2 is typically expressed on circulating bone marrow-derived endothelial progenitor cells and on vascular endothelial cells [15]. The signalling transduction arising from the binding between VEGFR-2 and VEGF molecules involves an initial dimerization of the receptor, which is followed by an auto-phosphorylation event taking place on the carboxy-terminal TK domain of the receptor itself. Subsequently, several different molecular pathways are simultaneously activated (Fig. 1): the Raf/MEK/Erk pathway, which gives rise to endothelial cell proliferation, the p38-MAPK pathway, which is related to endothelial cell migration, and the PI3K/AKT/mTOR pathway, which is responsible for the enhanced survival of endothelial cells and for the increased vascular permeability [16,17]. Although the binding between VEGF and VEGFR-2 is characterized by lower affinity than what observed with VEGFR-1 and the same ligand, it gives rise to a significantly more robust kinase activity [18]. As a dramatic result, an enhanced formation of blood vessels is observed, which translates into an increased microvessel density and a higher proliferation rate of vascular endothelial cells [15]. On behalf of the mentioned properties, VEGFR-2 is considered as the main responsible of tumour angiogenesis. Although it is associated with overall worse clinical outcomes, VEGFR-2 represents also an appealing target for novel anti-cancer therapies: as of today, it is possible to inhibit the signal arising from the activation of VEGFR-2 through several pharmacodynamics approaches, including receptor blockade (ramucirumab), seizure of the ligand (bevacizumab), and small-molecule inhibition (sorafenib, sunitinib, apatinib, cediranib, telatinib) [10].

Apatinib: preclinical and early-clinical data

Apatinib mesylate – a compound derived from valatinib – exhibits a particularly intriguing anti-tumour efficacy: the compound, formerly YN968D1 (N-[4-(1-cyano-cyclopentyl) phenyl]-2-(4-pyridylmethyl) amino-3-pyridine carboxamide mesylate), is a new inhibitor of VEGFR-2 tyrosine kinase targeting the intracellular ATP-binding site of the receptor, preventing phosphorylation and subsequent downstream signalling (Fig. 1). Apatinib has shown a superior in vivo efficacy compared to valatinib in xenograft models [19]. In 2010, a study investigated the antitumour activity of apatinib in both in vitro and in vivo models [12]. On in vitro models, apatinib elicited a suppression of the kinase activities of VEGFR-2, c-kit and c-src. Additionally, the compound inhibited the intracellular

Fig. 1. VEGFR-2 and VEGF molecular pathways.
phosphorylation of VEGFR-2, c-kit and PDGFRb; the in vitro inhibitory concentration to obtain a 50% of enzymatic inhibition (IC50) has been found to be lower for apatinib, then for what observed with other recently-developed anti VEGFR agents such as sunitinib (0.001 versus 0.005, respectively) [12,20]. In in vivo models, the effects of apatinib have been evaluated with regard to the inhibition of tumour growth on several established human tumour xenograft models, including GC. In these models, apatinib alone and in combination with chemotherapeutic agents showed a clear anti-tumour efficacy and a relatively safe profile. Intriguingly, the compound was able to block tumour growth in vivo models only, confirming that the observed antitumour activity was due to an exclusive effect on the angiogenic phenomenon [12].

Based on these results, a phase I study was performed to determine the maximum-tolerated dose (MTD), dose-limiting toxicities (DLT) and pharmacokinetic (PK) profiles of apatinib, and to set up a recommended dose of apatinib for the following phase II trials for patients with advanced solid tumours [21]. Additionally, the antitumour activity of the compound has been evaluated as well. Forty-six patients were enrolled in the study, with the most common tumour locations being located in the gastrointestinal tract. The dose escalation programme consisted of five levels of dose, ranging from 250 to 1000 mg. The MTD was determined as 850 mg once daily. The most frequent adverse events of apatinib were the same ones typical of other antiangiogenic agents, including hypertension in almost 70% of patients, proteinuria in about one half of patients, and hand–foot syndrome which has been observed in 46% of patients. Hypertension was generally easily manageable through antihypertensive agents as well as the hand–foot syndrome through the use of local treatment. The dose-limiting toxicities observed with 1000 mg administrations were hypertension and hand–foot syndrome. PK analysis has been performed in 27 patients. This analysis showed an early absorption with a half-life of 9 hours. In regard of response, thirty-seven patients were evaluable for best overall response. PR was noted in seven patients (18.9%), SD 24 (64.9%), with a disease control rate of 83.8% at 8 weeks. Among the 7 patients who achieved PR, the presence of a GC patient has been documented.

Clinical data

On the basis of the preclinical studies and phase I data, a subsequent phase II, randomized, double-blind, placebo-controlled trial was conducted [22,23]. This study involved heavily pre-treated metastatic GC patients. The primary end-point of this study was PFS. One-hundred-forty-four patients were enrolled to receive placebo (group A), apatinib 850 mg once daily (group B), or apatinib 425 mg twice daily (group C). The percentages of patients receiving at least two cycles of treatment involved a 50% in the placebo group, 74.5% in group B, and 69.6% in group C. Apatinib significantly improved PFS when compared with placebo. PFS were 3.67 months and 3.20 months for patients who received apatinib 850 mg once daily and 425 mg twice daily, respectively, and 1.40 months for patients who received placebo. Additionally, in groups A, B, and C, the median OS values were 2.50 months, 4.83 months, and 4.27 months, respectively. The toxicities were tolerable or could be clinically managed. The most frequent grade 3 or grade 4 adverse events were documented as hand–foot syndrome and hypertension. Haematological toxicities were rarely noted. Fatigue was a common adverse, in total 10.4%, 17.0%, and 15.2% of patients of groups A, B and C developed fatigue. However, only approximately 2% of patients experienced a grade 3–4 [23].

Finally, a study is currently comparing apatinib (850 mg/daily) to placebo. This is randomized, phase III trial in third line of therapy for GC, with OS as the primary endpoint. Secondary endpoints involve PFS, overall response rate, quality of life, safety profile [24]. Preliminary results of this study were reported at the ASCO annual meeting 2014. The authors reported a median OS significantly longer in the apatinib group compared with that observed in the placebo group (195 days versus 140 days; HR = 0.71; 95% CI (0.54–0.94); P < 0.016). With regard to secondary endpoint, apatinib showed a longer PFS compared with the placebo group (78 days versus 53 days, HR = 0.44, 95% CI (0.33–0.61), P < 0.0001) and a better response rate of 2.84% versus 0.00% in favour of apatinib. Finally, apatinib was well tolerated, the grade 3/4 adverse occurred in more than 2% of patients. Confirming the results of the previous studies, hypertension, hand-and-foot syndrome, proteinuria, fatigue, anorexia, and elevatedaminotransferase were the most observed adverse events. Selected ongoing trials with apatinib in gastric cancer are summarized in Table 1.

Discussion and future directions

Targeting angiogenesis was shown to be effective in many types of cancer including lung, breast, brain, renal, and colon cancers [25,26]. However, the role of antiangiogenic agents is controversial in GC: in fact, if from one side ramucirumab has been the first anti-angiogenic agent approved for the treatment of metastatic GC patients, on the other hand several antiangiogenic drugs failed to show an improvement in terms of OS for the same kind of patients. The different efficacy of these agents may be due by the different clinical trial design and/or the selected patient population among the studies. It is well known that tumour site, histology and ethnic differences may influence the expression of potential antiangiogenic targets and the chosen backbone of chemotherapy may further influence the response to antiangiogenic treatment [10]. Unfortunately, the lack of specific predictive biomarkers will not allow an appropriate selection of treatment based on the peculiar patient characteristics.

However, angiogenesis plays a major role in GC development and progression. In fact, Cancer Genome Atlas Research Network (TCGA) has demonstrated how some subtypes of GC are associated with a recurrent amplification of the VEGF-A gene and with an elevated expression of the angiogenesis-related pathways [11,27,28]. As is well known, angiogenesis is mediated by vascular endothelial growth

### Table 1

<table>
<thead>
<tr>
<th>Clinical trial identifier</th>
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<th>Setting</th>
<th>Primary endpoint</th>
<th>Status</th>
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<td>IV</td>
<td>Second line</td>
<td>Safety and efficacy</td>
<td>Ongoing but not recruiting</td>
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<tr>
<td>NCT02409199</td>
<td>II/III</td>
<td>Second line</td>
<td>Progression free survival</td>
<td>Recruiting</td>
</tr>
<tr>
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<td>Recruiting</td>
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<tr>
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<td>Disease free survival</td>
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<tr>
<td>NCT02509806</td>
<td>II/III</td>
<td>Maintenance therapy after first-line chemotherapy</td>
<td>Progression free survival</td>
<td>Not yet open for recruiting</td>
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<tr>
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<td>First line</td>
<td>Progression free survival; safety</td>
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<tr>
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<td>Radical resection rate</td>
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</tr>
<tr>
<td>NCT02586256</td>
<td>II/III</td>
<td>Second line</td>
<td>Progression free survival</td>
<td>Not yet open for recruiting</td>
</tr>
</tbody>
</table>

* Patients converted from an initially unresectable GC to a resectable cancer.
factor (VEGF) [29]. The activity of VEGF is mediated by its binding to three membrane receptor tyrosine kinases (RTK): VEGF-R-1 (Flt1); VEGF-2 (KDR); and VEGF-3 (Flt4) [14]. Among these, VEGFR2 has a key role because it is widely considered to be the primary receptor mediating angiogenesis [29]. VEGFR2, mainly expressed on endothelial cells, is principally responsible for mediating the mitogenic, angiogenic and permeability-enhancing effects of VEGF [14,30]. Therefore, VEGFR2, the blockade of VEGFR2, could be a promising strategy to inhibit tumour-induced angiogenesis. Additionally, the expression of VEGFR2 is a prognostic factor correlated with poor prognosis in GC [31]. The strategies to neutralize the VEGFR2 can include antibodies against to VEGF or portion of VEGFR receptor; or small molecule inhibitor of kinase activity of VEGFR. In this contest, although the first evidence of efficacy of apatinib in GC is very small (one phase II trial and preliminary results of a phase III study), it should be underlined that the patients of these studies consisted of patients who did not respond or who experienced pathological progression with second-line chemotherapy. Unfortunately, these patients in the third line of treatment have generally poor results, with response rate estimated to be as low as 10% and without any evidence of prolonged survival [32,33]. Therefore, in order to state definitive conclusions, the final results of the phase III trial should be awaited; in the meanwhile, apatinib has been approved by China Food and Drug Administration (CFDA) in December 2014 for patients with pre-treated metastatic GC. Additional information about apatinib will be available from the ongoing phase IV study (NCT02426034) started in April 2015 focused on its safety and efficacy in second line of treatment in clinical practice (a total of 2000 patients are planned) and from the NCT02409199 trial. This study will be the first to randomize apatinib with another drug. In this study, the efficacy and safety of apatinib will be compared to docetaxel-based treatment in patients with advanced GC. To date, this study is currently recruiting participants. In addition, the oral administration and the relative safety of apatinib opened the way to several studies in which apatinib is provided as maintenance therapy after the first line of treatment (NCT02537171; NCT02501469; NCT2509806). Furthermore, considering the limited benefit of apatinib as a single agent, several studies are investigating its combination with chemotherapy. Three ongoing studies will evaluate (1) the efficacy and safety of apatinib combined with S-1 as first-line therapy for patients with advanced gastric or gastro-oesophageal junction adenocarcinoma (NCT02525237), (2) the efficacy and safety of S1/docetaxel chemotherapy plus apatinib in the conversion therapy of metastatic GC (NCT02529878) (i.e. patients converted from an initially unresectable GC to a resectable cancer) and (3) the efficacy and safety of Apatinib plus docetaxel versus docetaxel as second-line treatment in advanced GC (AHEAD-301 trial (NCT02596256)).

With regard to the adverse events, fatigue is the most common side effect among patients treated with apatinib. However, only approximately 2% of patients experienced a grade 3 to grade 4 fatigue. Probably, the favourable safety profile of apatinib could be related to the selective inhibition of VEGFR2 [23]. Interestingly, although apatinib has been tested at 850 mg once daily and 425 mg twice daily, patients treated with apatinib as a once-daily regimen had fewer grade 3 to 4 adverse events than those given twice daily [23]. Unfortunately, no study reported a correlation between the appearance of adverse event, as a possible surrogate markers and response to apatinib.

Because the aim of targeted drug therapy is to maintain target inhibition continuously over the duration of treatment, and the most convenient regimen is once a day oral dosing, a plasma half-life of 8–12 hours is most desirable. Shorter half-lives may require multiple daily doses, whereas longer half-lives will lead to increasing drug levels in plasma (a steady state will be achieved after three to five half-lives) and will also increase the potential for prolonged periods of toxicity. In case of Apatinib with its 9 hours half-life give once/daily, it represents a promising drug as an adequate steady state, precise downregulation of its VEGFR2 target along with a low rate of toxicity. Similar molecules acting with a short half-life as apatinib already registered are Axitinib with 2.5–6.1 hours or Dasatinib 3–5 h [34,35].

Conclusions

To date, with the exception of trastuzumab in HER2-positive GC and ramucirumab, several agents targeting different pathways, such as EGFR, mTOR and c-Met, have not been shown to improve survival [36–42]. However, there is an urgent need to improve the treatment of metastatic GC patients.

As reported in a recent meta-analysis [43] of 22 trials exploring targeted therapy for a total of 7022 advanced GC patients demonstrating positive results for antiangiogenic agents in terms of OS (HR 0.759; P < 0.001), it would be plausible to sustain that the angiogenesis process is an important driver of the gastric diseases and it can be an optimal target in GC.

However, parallel to the drug development, it would be also important to identify predictive biomarker to help in selecting the optimal candidates to antiangiogenic-based therapy.

Future efforts in research are awaited to gain our knowledge about novel oral VEGFR2 inhibitors in order to improve the outcome of GC patients and to better define good responders.

Conflict of interest

The authors declare that they have no conflict of interest.

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