

# Critical analysis of the potential for therapeutic targeting of mammalian target of rapamycin (mTOR) in gastric cancer

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**Abstract:** Multidisciplinary treatment including chemotherapy has become the global standard of care for patients with metastatic gastric cancer (mGC); nonetheless, survival remains poor. Although many molecular-targeted therapies have been developed for various cancers, only anti-HER2 treatment has produced promising results in patients with mGC. Mammalian target of rapamycin (mTOR) plays a key role in cell proliferation, antiapoptosis, and metastasis in signaling pathways from the tyrosine kinase receptor, and its activation has been demonstrated in gastric cancer (GC) cells. This review discusses the clinical relevance of mTOR in GC and examines its potential as a therapeutic target in patients with mGC. Preclinical studies in animal models suggest that suppression of the mTOR pathway inhibits the proliferation of GC cells and delays tumor progression. The mTOR inhibitor everolimus has been evaluated as second- or third-line treatment in clinical trials. Adverse events were well tolerated although the effectiveness of everolimus alone was limited. Everolimus is now being evaluated in combination with chemotherapy in Phase III clinical studies in this subgroup of patients. Two Phase III studies include exploratory biomarker research designed to evaluate the predictive value of the expression or mutation of molecules related to the Akt/mTOR signaling pathway. These biomarker studies may lead to the realization of targeted therapy for selected patients with mGC in the future.

**Keywords:** gastric cancer, mTOR, everolimus

## Introduction

Gastric cancer (GC) is the fourth most common cancer, with 989,600 newly diagnosed cases worldwide in 2008, accounting for about 8% of all newly diagnosed cancers.<sup>1</sup> GC is the second leading cause of cancer-related mortality, with 738,000 deaths per year. The outcomes of GC remain poor, with an estimated relative 5-year survival rate of 25% in Europe.<sup>2</sup> In contrast, the 5-year survival rate was 68.9% in a study of 13,626 patients treated in Japan in 2002.<sup>3</sup> The higher survival rate in Japan is attributed to early detection of GC by cancer screening programs and aggressive dissection of regional lymph nodes by advanced surgical techniques. At present, the treatment of choice for advanced GC is complete surgical removal of the tumor and adjacent lymph nodes. Nonetheless, many patients with advanced disease have recurrence, probably caused by the presence of occult micrometastasis unable to be managed by surgery alone. Multidisciplinary treatment strategies including surgery with chemotherapy or radiotherapy prolong survival; however, the benefits of therapeutic approaches such as chemotherapy and radiotherapy remain very limited in far advanced GC.

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## Current chemotherapy with cytotoxic agents for GC

Median overall survival time (MST) was 10 to 13 months in patients with metastatic or unresectable gastric cancer (mGC) who received chemotherapy including multiple cytotoxic agents.<sup>4-6</sup> S-1 is an oral fluoropyrimidine preparation consisting of tegafur, 5-chloro-2,4-dihydropyrimidine, and potassium oxonate. The results of three Phase III studies comparing S-1-based combination chemotherapy with S-1 monotherapy in Japan have suggested that cisplatin is the best partner for S-1. In the SPIRITS study, chemotherapy-naïve patients with mGC were randomly assigned to receive either S-1 plus cisplatin or S-1 alone.<sup>4</sup> The MST was significantly longer in the S-1 plus cisplatin group than in the S-1 alone group (13.0 versus 11.0 months,  $P=0.04$ ). The TOP-002 study was conducted to compare the effectiveness of S-1 plus irinotecan with that of S-1 alone in patients with previously untreated advanced GC.<sup>5</sup> The S-1 and irinotecan combination was not significantly superior to S-1 alone in terms of overall survival, but it was associated with longer MST than S-1 alone (12.8 versus 10.5 months,  $P=0.23$ ). The START study compared S-1 plus docetaxel with S-1 alone in patients with mGC.<sup>6</sup> S-1 plus docetaxel was shown to be significantly superior to S-1 alone (MST, 12.5 versus 10.8 months,  $P=0.04$ ). The FLAGS trial was conducted in Western countries, and patients with mGC were assigned to receive either cisplatin plus S-1 or cisplatin plus infusional fluorouracil.<sup>7</sup> In Japan, S-1 has been administered in a daily dose of 80 mg/m<sup>2</sup> of body surface area in many clinical trials. However, a lower daily dose of 50 mg/m<sup>2</sup> was used in the FLAGS study because diarrhea occurred as dose-limiting toxicity in initial clinical trials performed in Western countries.<sup>8</sup> In the FLAGS study, overall survival did not differ significantly between the patients who received cisplatin plus S-1 and those who received cisplatin plus infusional fluorouracil (8.6 versus 7.9 months,  $P=0.20$ ); however, cisplatin plus S-1 had significant safety advantages in terms of treatment-related deaths and adverse events such as neutropenia, complicated neutropenia, and stomatitis. S-1 is therefore considered an alternative to infusional fluorouracil, even in Western countries.

Capecitabine is another oral fluoropyrimidine that is activated in tumor tissue by a three-step enzymatic conversion, the final step of which requires thymidine phosphorylase. The REAL2 trial was designed to determine whether fluorouracil could be replaced by capecitabine and cisplatin be replaced by oxaliplatin in the triplet regimen of epirubicin, cisplatin, and infused fluorouracil, which had been widely used to treat mGC in Europe.<sup>9</sup> The MST of the patients who received

combined chemotherapy with epirubicin, oxaliplatin, and capecitabine (EOC) was significantly longer than that of the patients who received epirubicin, cisplatin, and infused fluorouracil (11.3 versus 9.9 months,  $P=0.02$ ).

Triplet regimens including docetaxel have also been developed. The V325 trial compared the therapeutic usefulness of docetaxel, cisplatin, and fluorouracil (DCF) with that of cisplatin and fluorouracil (CF) in the Phase III part of the study in patients with mGC.<sup>10</sup> The MST was significantly longer in the DCF group than in the CF group (9.2 versus 8.6 months,  $P=0.02$ ). However, DCF was more frequently associated with toxic effects such as febrile neutropenia, severe diarrhea, and severe neurosensory impairment. Because these regimens were poorly tolerated clinically and required modification, a triplet regimen of docetaxel, cisplatin, and S-1 was developed and used to treat mGC in a Japanese Phase III trial.<sup>11</sup>

## Receptor tyrosine kinase-targeting agents for GC

Activated receptor tyrosine kinase (RTK) regulates many key signaling pathways participating in cell growth, survival, organ morphogenesis, vascularization, and tissue repair and regeneration.<sup>12</sup> RTK activity is strictly regulated in normal cells, whereas dysregulation or constitutive activation of RTK has been found in various cancers. Deregulated activation of RTK signaling can be caused by gene mutation, gene amplification, and overexpression of both receptor and ligand, which has been shown to correlate with the progression of various human cancers. In GC, high gene or protein expression of many RTKs including epidermal growth factor receptor (EGFR),<sup>13</sup> human epidermal growth factor receptor 2 (HER2),<sup>14,15</sup> vascular endothelial growth factor receptor (VEGFR),<sup>16,17</sup> and c-mesenchymal-epithelial transition (c-MET)<sup>18,19</sup> correlate with tumor progression or patient survival. Since various RTKs are involved in many aspects of tumor progression, these enzymes are considered promising therapeutic targets. Agents targeting RTK signaling pathways include antagonists of the ligands binding RTKs, inhibitors of receptor activation such as monoclonal antibodies, tyrosine kinase inhibitors (TKI), and inhibitors of downstream pathways.

Trastuzumab, an anti-HER2 monoclonal antibody, was the first RTK-targeting agent approved for the indication of GC worldwide. In the ToGA study, a Phase III trial performed in patients with advanced GC, the MST was significantly longer in patients who received trastuzumab plus chemotherapy (cisplatin and fluorouracil or capecitabine) than in patients who received chemotherapy alone

(13.8 versus 11.1 months,  $P=0.0046$ ).<sup>20</sup> Eligible patients in the ToGA study had advanced GC with either strongly positive (3+) HER2 expression on immunohistochemical (IHC) analysis or positive HER2 expression on fluorescence in situ hybridization. An exploratory analysis according to HER2 status suggested that overall survival was longer in patients with high expression of HER2 protein than in patients with low expression (16.0 versus 11.8 months,  $P=0.0002$ ).

Two agents targeting EGFR, a member of the HER family along with HER2, provided no additional benefits as compared with chemotherapy alone in Phase III clinical trials. In the EXPAND trial evaluating the efficacy of cetuximab, a monoclonal antibody of EGFR, the MST of patients assigned to cetuximab plus chemotherapy (capecitabine and cisplatin) was similar to that of patients assigned to chemotherapy alone (9.4 versus 10.7 months,  $P=0.95$ ).<sup>21</sup> In the REAL3 study of panitumumab, another monoclonal antibody of EGFR, the MST of patients who received panitumumab plus modified-dose EOC was significantly inferior to that of patients who received EOC alone (8.8 versus 11.3 months,  $P=0.013$ ); however, the lower doses of cytotoxic agents in the panitumumab plus modified-dose EOC group might have diminished effectiveness.<sup>22</sup> No biomarker was identified, and *KRAS* or *BRAF* mutations, which are associated with resistance to anti-EGFR treatment in colon cancer, were rarely found in that study. Lapatinib, a dual TKI against EGFR and HER2, also did not provide any additional benefit as second-line chemotherapy in patients with mGC.<sup>23</sup>

Bevacizumab is a monoclonal antibody targeting VEGF-A, which activates VEGFR-1 and VEGFR-2. In the AVAGAST trial, a Phase III study in patients with mGC, the MST of patients assigned to bevacizumab plus toxic chemotherapy (fluoropyrimidine plus cisplatin) was not significantly superior to that of patients assigned to toxic chemotherapy alone (12.1 versus 10.1 months,  $P=0.10$ ), although progression-free survival (PFS) was significantly prolonged by concurrent treatment with bevacizumab (6.7 versus 5.3 months,  $P=0.004$ ).<sup>24</sup> In the biomarker evaluation substudy of the AVAGAST trial, high plasma vascular endothelial growth factor (VEGF)-A levels and low tumor neuropilin-1 expression were associated with trends toward improved overall survival, but only in non-Asian patients.<sup>25</sup> Ramucirumab is a monoclonal antibody targeting the extracellular domain of VEGFR-2. In the REGARD study, a Phase III trial of second-line chemotherapy for mGC, ramucirumab prolonged overall survival as compared with best supportive care (MST 5.2 versus 3.8 months,  $P=0.047$ ).<sup>26</sup>

Rilotumumab is a monoclonal antibody designed to inhibit the hepatocyte growth factor (HGF) binding c-MET pathway. In a Phase II study in mGC, the additive effect of rilotumumab was clinically significant among patients with high c-MET expression.<sup>27</sup> In that study, c-MET expression did not frequently overlap with HER2 status, suggesting that c-MET inhibitors can be effective against tumors without HER2 expression.

Foretinib, a multikinase inhibitor of c-MET, VEGFR2, and three other receptors, lacked efficacy in Phase II studies of patients with mGC.<sup>28</sup> Sorafenib is an oral multitargeted TKI that inhibits VEGFR-1, VEGFR-2, VEGFR-3, platelet derived growth factor receptor (PDGFR), B-Raf, Raf-1, and c-Kit. A Phase II study of sorafenib combined with docetaxel and cisplatin resulted in survival similar to that obtained in other studies of chemotherapy alone.<sup>29</sup> Sunitinib is also a multitargeted TKI targeting rearranged during transfection (RET), VEGFR-1, VEGFR-2, VEGFR-3, PDGFR $\alpha$ , PDGFR $\beta$ , FMS-like tyrosine kinase-3 (Flt3), c-KIT, and colony-stimulating factor receptor. Sunitinib has been evaluated as monotherapy in two Phase II studies but was not effective as second-line treatment for mGC.<sup>30,31</sup>

## Introduction to mammalian target of rapamycin

Mammalian target of rapamycin (mTOR) has been recognized as a key regulator of cell growth, proliferation, metabolism, and angiogenesis.<sup>32–34</sup> mTOR functions by integrating extracellular signals, such as growth factors and hormones, with amino acid availability and intracellular energy status to control translation rates and additional metabolic processes.<sup>35</sup> Abnormal mTOR signaling has been associated with numerous pathological conditions, including cancer, immune disorders, diabetes mellitus, and cardiovascular and neurological diseases.<sup>36</sup>

mTOR exists as part of two functionally distinct protein complexes, mTORC1 and mTORC2. mTORC1 consists of mTOR catalytic subunit and two other proteins, regulatory-associated protein of mTOR (raptor) and mammalian lethal with SEC13 protein 8 (mLST8), the latter of which is also known as G protein beta subunit-like.<sup>37–39</sup> Raptor might have roles in mTORC1 assembly, recruiting substrates to mTORC1, and in regulating mTORC1 activity.<sup>40,41</sup> The strength of the association between mTOR and raptor is regulated by nutrients and other signals that control the mTORC1 pathway. Signaling from growth factors is mediated to mTORC1 via the PI3K/Akt pathway. The tuberous sclerosis complex (TSC1 and TSC2) is degraded by Akt, permitting mTORC1 activation. mTORC1 promotes protein synthesis via downstream

effectors, such as the regulators of ribosomal S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1).<sup>42,43</sup> mTORC1-activated S6K1 promotes ribosomal protein translation and is an important regulator of cell size. 4E-BP1 is inactivated by mTORC1, and release of eIF4E from 4E-BP1 enables the formation of the active eIF4F complex, which is required for cap-dependent translation of mRNA. mTORC2 contains mTOR and mLST8 as well as two other proteins, rapamycin-independent companion of mTOR (rictor) and mSin1 (also known as mitogen-activated protein-kinase-associated protein), instead of raptor.<sup>44,45</sup> Both rictor and mSin1 are necessary for phosphorylation of Akt, and then mTORC2 activates the positive feedback loop. Unlike raptor, the interaction between rictor and mTOR does not seem to be regulated by upstream signals. On the other hand, mTORC1-mediated S6K1 activation can inhibit mTORC2 through a negative feedback loop, thereby suppressing Akt activation.<sup>46,47</sup> mTORC1 inhibits uncoordinated 51-like kinase 1 (ULK1) and ULK2, linked to starvation-induced autophagy. mTORC1 interrupts the binding of mammalian autophagy-related protein 13 to ULK, thereby inhibiting the phosphorylation of the focal adhesion kinase-family interacting protein, FIP200, and inducing autophagy.<sup>48</sup>

The location of mTOR may have an important role in regulation of its signaling pathway. A nuclear transport signal in mTOR is critical for its downstream cytoplasmic signaling to S6K1.<sup>49</sup> The rictor and mSin1 components of mTORC2 were translocated from the nucleus to the cytoplasm by treatment with rapamycin, an mTOR inhibitor, in human fibroblasts.<sup>50</sup>

### Clinical significance of mTOR expression in GC

We summarize the expression of phosphorylated mTOR (p-mTOR) or mTOR in GC on IHC analysis and clinical outcomes in Table 1. We previously reported that the localization of p-mTOR on IHC analysis is differentially related to tumor progression and patient survival in GC.<sup>51</sup> The cytoplasmic expression of p-mTOR in GC cells was significantly associated with tumor depth, lymph-node metastasis, tumor stage, and poor survival while the nuclear expression of p-mTOR was significantly associated with favorable survival. Similar results were obtained in several studies. The expression of p-mTOR on IHC analysis was significantly associated with lymph-node metastasis and tumor stage in a larger study, and p-mTOR detected in cytoplasm or cell membrane was an independent prognostic factor.<sup>52</sup> However, overexpression was defined as staining positivity higher than that of normal

Table 1 mTOR expression (phosphorylated or not) on immunohistochemistry and clinical outcomes

	n	TMA	Positive or overexpression definition	%	Relation to clinico-pathological factors	Relation to survival	Reference
p-mTOR	1,072	Yes	Stronger staining than normal tissue	47	N, stage	Worse on UA and MA	52
	181	No	Weak intensity >20% of cells, or strong intensity >10%	51	N, stage	Worse on UA and MA**	53
mTOR	109	No	Cytoplasmic staining >10% of cells	63	T, N, stage, recurrence	Worse on UA, not associated with MA	51
	290*	Yes	Weak intensity >25% of cells or moderate or strong intensity	45	N, differentiated type	Not associated on UA and MA by the expression in the primary site but worse on UA and MA by the expression in N	54
	1,072	Yes	Stronger staining than normal tissue	51	Differentiated type, T, stage (inversely)	Better on UA, not associated with MA	52
	412	Yes	Any staining	62	Intestinal type	Better on UA and MA	58
	33	No	Cytoplasmic staining, any staining	52	T*, N, stage, differentiated type	Not evaluated	57

Notes: \*Represents patients with pathological subserosal invasion; \*\*represents not significant, but P-value <0.1. Abbreviations: MA, multivariate analysis; mTOR, mammalian target of rapamycin; N, metastatic lymph nodes; p-mTOR, phosphorylated mTOR; T, depth of tumor invasion; TMA, tissue microarray; UA, univariate analysis.



tissue in that study. Similar results were also obtained in another study, which reported that p-mTOR was detected mainly in cytoplasm and partly in cell membrane.<sup>53</sup> In that study, positive staining was based on a scoring system in which staining intensity was multiplied by the percentage of positive tumor cells. An et al showed that p-mTOR expression on IHC analysis was associated with the extent of lymph-node metastasis in GC with subserosal invasion; moreover, p-mTOR expression in metastatic lymph nodes correlated with poor disease-free survival.<sup>54</sup> Positive status of p-mTOR in cytoplasm or cell membrane was defined according to a scoring system based on staining intensity and extensity in that study. Lang et al reported that positive staining for p-mTOR was predominantly found at the tumor invasive front in GC, whereas the adjacent normal gastric mucosa mainly stained negative for p-mTOR.<sup>55</sup> Feng et al demonstrated cytoplasmic and membranous p-mTOR expression in GC, and positive p-mTOR expression was found even in chronic gastritis and intestinal metaplasia, which are considered precarcinomatous conditions.<sup>56</sup>

Nonactivated mTOR expression significantly correlated with lymph-node metastasis and pathological stage in GC.<sup>57</sup> However, conflicting results have been obtained in other larger studies. mTOR expression was significantly associated with favorable survival in one study.<sup>58</sup> Another study similarly reported that mTOR expression was more frequently detected in early-stage than in advanced-stage disease and was significantly associated with favorable survival.<sup>53</sup> In addition, nuclear expression of phosphorylated S6K1, which is activated by p-mTOR and participates in promotion of tumor progression, was associated with favorable outcomes in another study.<sup>58</sup> These inconsistent results suggest that localization of S6K1 might have an inhibitory role in tumor progression, but this remains to be confirmed.

## Preclinical studies of mTOR inhibition in GC cells

The downstream targets of mTOR, such as phosphorylated S6K1 and phosphorylated 4E-BP1, were decreased by mTOR inhibitors such as rapamycin or everolimus (RAD001) in some GC cell lines.<sup>59–63</sup> Everolimus reduced tumor vascularization and cell proliferation independently of signal transducer and activator of transcription 3, another downstream target of RTKs, in a mouse model.<sup>64</sup> Everolimus, a specific mTORC1 inhibitor, significantly reduced peritoneal dissemination in a xenograft model.<sup>65</sup> However, the basal phosphorylation level of 4E-BP1 was associated with sensitivity to everolimus in GC cell lines and a xenograft model.<sup>66</sup> In

addition, rapamycin upregulated insulin-like growth factor-1 receptor expression in a GC cell line, and rictor indeed played an important role in that mechanism.<sup>60</sup> A combination of rapamycin or everolimus with 5-fluorouracil displayed synergistic growth-inhibitory activity and downregulated thymidylate synthase in some GC cell lines.<sup>63,67,68</sup> In addition, inhibition of mTOR is a key molecular event in enhancing fluorouracil-induced apoptosis in some HER2-amplified GC cell lines, regardless of sensitivity to trastuzumab.<sup>69</sup> Inhibition of mTOR by everolimus counteracted the effects of VEGF induction by sunitinib, significantly reducing tumor in a GC xenograft model.<sup>62</sup> Interestingly, Ji et al demonstrated that everolimus and an Akt inhibitor induced beclin-1 expression and activated autophagic cell death pathway by extracellular signal-regulated kinases.<sup>70</sup>

## Critical trials of mTOR-targeted treatment in GC

Everolimus has been investigated in several clinical trials of mGC, and the critical trials are summarized in Table 2. In a Phase I dose-escalation (2.5, 5, or 10 mg/day) study of everolimus alone in nine patients with advanced solid tumors, including GC, dose-limiting toxicity (DLT) occurred, and the maximum-tolerated dose and recommended dose (RD) were determined to be 10 mg/day. One patient with GC markedly responded to 10 mg/day of everolimus.<sup>71</sup> In patients with esophagogastric cancer who had previously received chemotherapy, a Phase I dose-escalation study of everolimus (5, 7.5, or 10 mg/day) plus intravenous mitomycin C (5 mg/m<sup>2</sup>, every 3 weeks) reported one case of DLT at 10 mg/day in the extended portion of the trial. The maximum-tolerated dose and RD of everolimus were therefore estimated to be 10 mg/day. The most frequent grade 3 or higher adverse events were leukopenia (19%) and neutropenia (19%). Three patients (19%) had a partial response, and four (25%) had stable disease. Antitumor activity was highest in the 10 mg/day cohort.<sup>72</sup>

In a Phase II trial in Japan, everolimus (10 mg/day) was administered to 53 patients with mGC previously treated by chemotherapy.<sup>73</sup> The disease control rate, the endpoint of that study, was 56%, although all of these patients had stable disease, with no complete or partial response to treatment. The MST was 10.1 months, and the median PFS was 2.7 months. These results suggested that the primary benefit of everolimus was disease stabilization in mGC. Twenty-four patients (45%) had grade 3 or 4 adverse events, including anemia (9.4%), hyponatremia (9.4%), elevated gamma-glutamyltransferase levels (7.5%), and lymphopenia (7.5%).

**Table 2** Clinical trials of everolimus against metastatic gastric cancer

Phase	Treatment arm	Chemotherapy line	n	Main endpoint	Results	Reference
I	Everolimus	2nd or more	9	MTD	RD and MTD was 10 mg/day	71
I	Everolimus + mitomycin C	2nd or more	16	MTD	RD and MTD of everolimus was 10 mg/day	72
I	Everolimus + capecitabine	3rd or more	15	MTD	RD and MTD of everolimus was 10 mg/day	75
II	Everolimus	2nd, 3rd	53	DCR	DCR 56% (no CR/PR) Median OS 10.1 M, median PFS 2.7 M	73
II	Everolimus	2nd	54	ORR/DCR	ORR 3.9% (no CR), DCR 39% Median OS 8.3 M, median PFS 1.7 M	74
II	Everolimus + capecitabine	3rd or more	47	ORR	ORR 11% (no CR), DCR 49% Median OS 4.9 M, median PFS 2.6 M	76
III	Everolimus versus placebo (GRANITE-1)	2nd, 3rd	656	OS	ORR 4.5% versus 2.1% Median OS 5.4 M versus 4.3 M Median PFS 1.7 M versus 1.4 M	77

**Abbreviations:** CR, complete response; DCR, disease control rate; M, months; MTD, maximum-tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; RD, recommended dose.

In a Phase II trial performed in Korea, everolimus (10 mg/day) was administered to 44 patients who had mGC with no response to fluoropyrimidine or platinum therapy.<sup>74</sup> Two patients (3.7%) had a partial response, and the disease control rate was 39%. The MST was 8.3 months, and the median PFS was 1.7 months. In that study, the expressions of p-mTOR, S6K1, and 4E-BP1 were evaluated on IHC analysis as biomarkers. pS6<sup>Ser240/4</sup> was suggested to be a predictive biomarker because it was significantly associated with worse PFS and was moderately associated with overall survival.

A combination of everolimus plus capecitabine was given as third-line or subsequent chemotherapy to patients with mGC in a Korean study.<sup>75</sup> In this Phase I study, the RDs of everolimus and capecitabine were respectively estimated to be 10 mg/day and 1,300 mg/day (on days 1–14, every 3 weeks), although multiple DLT associated with grade 3 adverse effects, including hyperglycemia, hyponatremia, stomatitis, thrombocytopenia, and hypophosphatemia, occurred at the upper dose level. In a Phase II study of 47 patients with mGC, including 43 patients assessable for treatment response, five patients (11%) had a partial response, and 18 (38%) had stable disease.<sup>76</sup> The MST and median PFS were 4.9 and 2.6 months, respectively.

However, a Phase III study (GRANITE-1) of everolimus resulted in unfavorable outcomes as second- or third-line therapy.<sup>77</sup> Six hundred fifty-six patients with mGC were randomly assigned to everolimus 10 mg/day or to matching placebo (assignment ratio: 2:1), both given with best supportive care. MST was 5.4 months with everolimus and 4.3 months with placebo ( $P=0.124$ ) although median PFS was 1.7 months and 1.4 months in the everolimus group and placebo group, respectively ( $P<0.001$ ).

## Clinical trials of mTOR-targeted treatment in various cancers

The clinical efficacy of everolimus, an mTOR inhibitor, was initially demonstrated in a worldwide Phase III study in patients with renal cell carcinoma.<sup>78</sup> In that study, patients with metastatic renal cell carcinoma who had progressive disease while receiving sunitinib, sorafenib, or both were randomly assigned in a two-to-one ratio to receive everolimus (10 mg/day) or placebo in conjunction with best supportive care. Treatment with everolimus prolonged PFS as compared with placebo (median PFS 4.0 versus 1.9 months,  $P<0.0001$ ). Adverse events of grade 3 or 4 were significantly more frequent in the everolimus group and included stomatitis (3%) and hypercholesterolemia (12%).

Another mTOR inhibitor, ridaforolimus, was effective against sarcoma in a Phase III study.<sup>79</sup> Patients with metastatic soft tissue or bone sarcomas who had an objective response or stable disease during prior chemotherapy were randomly assigned to receive ridaforolimus (40 mg for 5 days every week) or placebo. Ridaforolimus treatment significantly improved PFS as compared with placebo (4.1 versus 3.4 months,  $P=0.001$ ) while the MST in the ridaforolimus and placebo groups was 21.1 and 19.9 months, respectively ( $P=0.46$ ).

However, disappointing results of monotherapy with mTOR-targeted agents have been reported in several types of cancers despite feasible adverse effects. In a Phase II study of everolimus (10 mg/day) in patients with gemcitabine-refractory metastatic pancreatic cancer, no response was noted, and only 21% of patients had stable disease.<sup>80</sup> The MST and median PFS were 4.5 and 1.8 months, respectively. In other Phase II studies of temsirolimus (25 mg/week) or everolimus (30 mg/week) in pancreatic cancer, most of the patients given either of these

mTOR inhibitors had received prior chemotherapy.<sup>81</sup> The study of temsirolimus was terminated because of clinically significant adverse effects. Everolimus produced no response or disease stability, with an MST of 2.9 months and a median PFS of 1.6 months in that study.

In small-cell lung cancer, everolimus (10 mg/day) had limited antitumor activity in previously treated patients, with an MST of 6.7 months and a median PFS of 1.3 months.<sup>82</sup> In that study, high phosphorylated Akt expression was modestly associated with overall survival, and baseline S6K1 expression was significantly higher in patients with disease control than in those with progression.

In a Phase II study of everolimus, unsatisfactory results were obtained in patients with hepatocellular carcinoma, most of whom had received prior chemotherapy.<sup>83</sup> The MST and median PFS were 8.4 and 3.8 months, respectively.

## Discussion

In Phase II clinical trials, a response to everolimus alone was rare among patients with mGC, although everolimus appeared to slightly prolong survival. However, a Phase III study (GRANITE-1) showed no efficacy of everolimus in terms of overall survival as compared with a placebo. Everolimus thus might not be effective as a single agent for mGC. As second-line monotherapy for gastric cancer, better responses to other cytotoxic agents, such as paclitaxel and irinotecan, have been obtained as compared with everolimus. In one Phase III study the response rate was 20.9% for paclitaxel and 13.6% for irinotecan.<sup>84</sup> Poor response to everolimus alone has been demonstrated in various malignancies; thus, everolimus was expected to maintain stable disease in Phase III studies. Even in a Phase III study that obtained favorable outcomes in renal cell carcinoma, the response rate was only 3%.<sup>78</sup> Poor response rates may be related to incomplete inhibition of the Akt/mTOR signaling pathway. In some preclinical studies, everolimus can incompletely inhibit the mTOR signaling pathway and suppress tumor cell proliferation by activation of insulin-like growth factor-1 receptor.<sup>60,70</sup>

However, adding everolimus to a standard regimen has not yet been shown to be beneficial. Combined chemotherapy with everolimus and a cytotoxic agent may improve the response rate as well as overall survival. In another Phase III study (AIO-STO-0111) of second- or third-line chemotherapy for gastric cancer, the effectiveness and safety of combined chemotherapy with everolimus plus paclitaxel are being compared with those of a placebo plus paclitaxel.<sup>85</sup> As mentioned above, clinical trials combining everolimus with capecitabine have obtained promising

results in patients with mGC refractory to chemotherapy.<sup>74</sup> In several studies of GC cells, combining an mTOR inhibitor with fluorouracil, another TKI, or an Akt inhibitor boosted antitumor activity.<sup>67–70</sup>

Everolimus has not been evaluated as first-line chemotherapy for mGC. In metastatic renal cell carcinoma, a randomized clinical trial was performed to compare everolimus with sunitinib as first-line chemotherapy. Unfortunately, patients who received everolimus had shorter PFS than those who received sunitinib.<sup>86</sup> The efficacy of everolimus as first-line monotherapy thus appears to be limited; therefore, combining everolimus with cytotoxic agents or other targeting agents is also needed for first-line chemotherapy.

mTOR inhibitors have been administered to unselected patients with mGC in clinical trials. Enhancement of effectiveness requires that mTOR inhibitors are given to patients whose tumors are regulated by mTOR-dependent signaling pathways. The tumor expression of S6K1 might be a potential predictor of the response to everolimus, although small clinical trials performed to date have yielded conflicting results. The aforementioned Phase III studies (GRANITE-1 and AIO-STO-0111) include exploratory biomarker research designed to evaluate the predictive value of the expression or mutation of molecules related to the Akt/mTOR signaling pathway. These biomarker studies may lead to the realization of targeted therapy for selected patients with mGC in the future.

## Disclosure

The authors report no conflicts of interest in this work.

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