Chemotherapy for advanced gastric cancer: future perspective in Japan

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Abstract Differences in clinical outcomes between advanced gastric cancer (AGC) in Asia and that in other regions have been discussed for a long time, although no major significant differences in molecular profiles have been reported. The anti-human epidermal growth factor receptor 2 antibody trastuzumab and the anti-vascular endothelial growth factor receptor 2 monoclonal antibody ramucirumab were both approved as a treatment for AGC on the basis of global phase 3 trials including Japan. In recent years, others new agents for treatment of AGC have been investigated in global or Asian studies. Randomized phase 2 trials in Japan showed a higher response rate to S-1 plus leucovorin and oxaliplatin than to standard S-1 plus cisplatin, which is the rationale for an ongoing phase 3 trial in Asia (SOLAR trial). A recent global phase 1 trial of the anti-programmed cell death 1 monoclonal antibody pembrolizumab showed similar efficacy results in Asian patients and non-Asian patients, which led to large global phase 2 and phase 3 studies. Although the perspective of treatment of AGC in the near future depends on the results of ongoing large clinical trials, individualized choice of treatment based on more detailed molecular information will become important.

Keywords Chemotherapy · Gastric cancer · Human epidermal growth factor receptor 2 · Immune checkpoint inhibitors · Vascular endothelial growth factor receptor 2

Introduction

Effective cytotoxic agents for unresectable advanced gastric cancer (AGC) or recurrent gastric cancer include 5-fluorouracil (5-FU), orally administered fluoropyrimidine, platinum agents, taxanes, irinotecan, and anthracyclines. A commonly used first-line therapy is the combination of fluoropyrimidine and platinum-based chemotherapy with or without docetaxel or anthracycline, which results in a median progression-free survival (PFS) of 5–6 months and a median overall survival (OS) of 10–15 months [1–4]. The survival benefit of second-line therapy was recently confirmed in several randomized trials [5–7]. Trastuzumab, a monoclonal antibody for human epidermal growth factor receptor 2 (HER2), significantly increased the OS of HER2-positive AGC patients [8]; thus chemotherapy plus trastuzumab therapy became standard care (Table 1). Ramucirumab, an anti-vascular endothelial growth factor receptor 2 (VEGFR2) antibody, is proven to be effective for previously treated AGC. The addition of ramucirumab therapy to best supportive care was associated with significantly increased OS of patients after disease progression with first-line chemotherapy [9]. Ramucirumab plus paclitaxel chemotherapy compared with paclitaxel alone also significantly prolonged OS of patients after first-line treatment [10].

Even after the introduction of these new agents, the prognosis of patients with AGC remains unsatisfactory; therefore the introduction of more effective treatments is desired. In this article, current knowledge of molecular profiles in gastric cancer and the status of development of chemotherapy for AGC are reviewed.

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Molecular profiles in gastric cancer: Asia versus the rest of the world

Since the establishment of HER2 as a treatment target in AGC, many findings have been reported on the expression of other growth factors or receptor tyrosine kinases (RTKs). A certain percentage of patients showed overexpression of epidermal growth factor receptor (EGFR), MET as a receptor of hepatocyte growth factor, and fibroblast growth factor receptor (FGFR) [11]. Detailed molecular profiles of gastric cancer have also been reported recently or are being evaluated in large-scale international cancer genome studies. In a study by The Cancer Genome Atlas Research Network [12], four subtypes of gastric cancers were described: (1) tumors positive for Epstein–Barr virus (EBV), (2) microsatellite instability (MSI)-high tumors, (3) genomically stable tumors, and (4) tumors with chromosomal instability. The subtype of EBV-positive cancer is characterized by frequent observations in gastric corpus, recurrent *PIK3CA* and *ARID1A* mutations, high amplification at chromosomal locus 9p including the programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) genes and high expression of PD-L1 and PD-L2, extreme DNA hypermethylation, etc., whereas the MSI-high subtype shows frequent mutation of multiple genes such as *HER3*. On the other hand, the genomically stable subtype has a few somatic copy-number alterations but involves *ARID1A* and *RHOA* mutations or *CLDN18-ARHGAP* gene fusions. The subtype with chromosomal instability is rich in *TP53* mutations, and has relatively numerous amplifications of RTK genes. Although The Cancer Genome Atlas Research Network study did not include tumor samples from Japan, a study of 121 Japanese patients with resected gastric cancer did not demonstrate much difference regarding gene mutations, with relatively high frequencies of *TP53*, *PIK3CA*, *ARID1A*, and *CDH1* mutations, and kinds of gene amplifications of RTKs and of those involved in the cell cycle [13]. Molecular targeting agents for particular genomic abnormalities such as MET overexpression, MET amplification or FGFR amplifications have been evaluated for AGC. Also, MSI-high tumors are very sensitive to immune check point inhibitors. Some of these studies will be discussed in following sections.

From the immunological viewpoint, non-Asian gastric cancers are reported to be associated with a significantly higher expression of several T-cell markers (CD3, CD45RO, CD8, etc.), whereas a higher expression of the immunosuppressive transcription factor FOXP3 was reported in Japanese gastric cancers [14]. On the other hand, PD-L1 expression was reported in both Western and Japanese patient cohorts [15, 16]. Both studies showed PD-L1 expression was more frequently observed in immune

cells than in tumor cells. The influence of these immunological factors on the efficacies of immune checkpoint inhibitors warrants further evaluation in clinical studies.

Ongoing development in the treatment of AGC

Various new agents have been investigated in large clinical trials for AGC (Tables 1, 2).

Cytotoxic chemotherapy

Nab-paclitaxel is nanoparticle-albumin-bound paclitaxel and does not contain Cremophor® EL or ethanol. As a result, nab-paclitaxel can reduce the risk of hypersensitivity reaction and can be administered to patients who are intolerant of alcohol. The ABSOLUTE trial is a Japanese phase 3 trial to compare the efficacy and safety of 3-weekly doses or weekly doses of nab-paclitaxel and weekly doses of Cremophor-based paclitaxel in patients with AGC refractory to first-line chemotherapy consisting of fluoropyrimidines [17]. In this study, the primary end point was achieved and showed noninferiority of weekly doses of nab-paclitaxel in terms of OS (Table 1). The median OS for weekly doses of nab-paclitaxel was 11.1 months and that for Cremophor-based paclitaxel was 10.9 months (hazard ratio 0.97, 97.5 % confidence interval 0.76–1.23, predefined noninferiority margin 1.25). The frequencies of adverse events were not significantly different between weekly doses of nab-paclitaxel and weekly doses of Cremophor-based paclitaxel. In contrast, noninferiority of nab-paclitaxel administered every 3 weeks compared with Cremophor-based paclitaxel in terms of OS was not confirmed. Moreover, nab-paclitaxel administered every 3 weeks was associated with a higher frequency of febrile neutropenia or peripheral sensory neuropathy.

A randomized phase 3 trial of S-1 plus leucovorin versus S-1 plus leucovorin plus oxaliplatin (SOL) versus S-1 plus cisplatin in AGC patients showed a higher response rate in the SOL group and longer OS [18]. The phase 3 SOLAR trial comparing TAS-118 (S-1 and leucovorin) plus oxaliplatin and S-1 plus cisplatin is ongoing in Asian countries (NCT02322593). TAS-102 is a novel oral nucleoside anti-tumor agent containing trifluridine and tipiracil hydrochloride, which prevents the degradation of trifluridine. In a pivotal phase 3 trial for colorectal cancer, TAS-102 showed OS benefit in comparison with placebo in patients with metastatic colorectal cancer who had been treated with standard chemotherapies [19]. A Japanese phase 2 trial of TAS-102 for pretreated AGC showed disease control rate of 65.5 % by investigator assessment [20]. The median PFS was 2.9 months and the median OS was 8.7 months. The

Table 1 Selected randomized phase 2 and phase 3 trials for advanced gastric cancer

Trial/authors	Phase	Line	Target	Regimens	No. of patients	Median OS	Median PFS	ORR (%)
ToGA [8]	3	1st	HER2 (with enrichment)	FP/XP + trastuzumab FP/XP	294 290	13.8 months 11 months HR 0.74, $p = 0.0046$	6.7 months 5.5 months HR 0.71, $p = 0.0002$	47 35
Logic [22]	3	1st	HER2 (with enrichment)	CapeOX + lapatinib CapeOX	272 273	12.2 months 10.5 months HR 0.91, $p = 0.35$	6 months 5.4 months HR 0.82, $p = 0.038$	53 39
TyTAN [23]	3	2nd	HER2 (with enrichment)	Paxlitaxel + lapatinib	132 129	11 months 8 months HR 0.84, $p = 0.21$	5.4 months 4.4 months HR 0.85, $p = 0.24$	27 9
GATSBY [24]	3	2nd	HER2 (with enrichment)	T-DM1 Paclitaxel, docetaxel	228 117	7.9 months 8.6 months HR 1.15, $p = 0.86$	2.7 months 2.9 months HR 1.13, $p = 0.31$	20.6 19.6
REAL-3 [25]	3	1st	EGFR	EOC + panitumumab EOC	276 266	8.8 months 11.3 months HR 1.37, $p = 0.013$	6 months 7.4 months HR 1.22, $p = 0.068$	46 42
EXPAND [26]	3	1st	EGFR	XP + cetuximab XP	455 449	9.4 months 10.7 months HR 1.00, $p = 0.95$	4.4 months 5.6 months HR = 1.09, $p = 0.32$	30 29
GRANITE-1 [27]	3	2nd/3rd	mTOR	Everolimus Placebo	439 217	5.39 months 4.34 months HR 0.90, $p = 0.1244$	1.68 months 1.41 months HR 0.66, $p < 0.0001$	4.5 2.1
RILOMET1 [28]	3	1st	HGF (with enrichment)	ECX + rilotumumab ECX + placebo	304 305	9.6 months 11.5 months HR = 1.36, $p = 0.021$	5.7 months 5.7 months HR = 1.27, $p = 0.025$	30 39.2
METGastric [29]	3	1st	MET (with enrichment)	FOLFOX + onartuzumab FOLFOX	121	11 months 11.3 months HR = 0.82, $p = 0.244$	6.7 months 6.8 months HR = 0.90, $p = 0.43$	46 41
AVAGAST [30]	3	1st	VEGF	XP + bevacizumab XP + placebo	387 387	12.1 months 10.1 months HR = 0.87, $p = 0.1002$	6.7 months 5.3 months HR = 0.80, $p = 0.0037$	46 37
REGARD [9]	3	2nd	VEGFR2	Ramucirumab Placebo	238 117	5.2 months 3.8 months HR 0.776, $p = 0.047$	2.1 months 1.3 months HR 0.483, $p < 0.0001$	3 3
RAINBOW [10]	3	2nd	VEGFR2	Paclitaxel + ramucirumab Paclitaxel + placebo	330 335	9.63 months 7.36 months HR 0.807, $p = 0.0169$	4.4 months 2.86 months HR 0.635, $p < 0.0001$	28 16

Table 1 continued

Trial/authors	Phase	Line	Target	Regimens	No. of patients	Median OS	Median PFS	ORR (%)
Li et al. [43]	3	3rd	VEGFR2	Apatinib Placebo	176 91	6.5 months 4.7 months HR 0.71, $p < 0.015$	2.6 months 1.8 months HR 0.44, $p < 0.0001$	1.7 0
PHOENIX-GC [21]	3	1st	Cytotoxic	S-1 + paclitaxel IV, IP SP	122 61	17.7 months 15.2 months HR 0.72, $p = 0.081$	NR NR	53 60
ABSOLUTE [17]	3	2nd	Cytotoxic	Nab-paclitaxel (weekly) Paclitaxel	240 243	11.1 months 10.9 months HR 0.97 (95 % CI 0.76–1.23)	5.3 months 3.8 months HR 0.88 (95 % CI 0.73–1.06)	32.7 24.3
				Nab-paclitaxel (every 3 weeks) Paclitaxel	243 243	10.3 months 10.9 months HR 1.06 (95 % CI 0.87–1.31)	3.8 months 3.8 months HR 1.03 (95 % CI 0.85–1.24)	25.3 24.3
Hironaka et al. [18]	2	1st	Cytotoxic	SOL S-1 + LV SP	47 47 48	18.4 months 15.6 months 12.6 months HR 0.59, $p = 0.023$ (SOL vs SP)	8.3 months 4.2 months 5.6 months HR 0.60, $p = 0.054$ (SOL vs SP)	66 43 46
Satoh et al. [31]	2	2nd	EGFR	Irinotecan + nimotuzumab Irinotecan	40 43	293 days 227 days HR 0.717, $p = 0.22$	85 days 73 days HR 0.86, $p = 0.5668$	18.4 10.3
INTEGRATE [44]	2	2nd or 3rd	VEGFR, RET, RAF	Regorafenib Placebo	97 50	25 weeks 19 weeks HR 0.68, $p = 0.065$	11 weeks 4 weeks HR 0.41, $p < 0.0001$	3 2
Bang et al. [47]	2	2nd	PARP	Paclitaxel + olaparib Pacelitaxel + placebo	62 62	13.1 months 8.3 months HR 0.56, $p = 0.01$	3.91 months 3.55 months HR 0.80, $p = 0.131$	26.4 19.1
Moehler et al. [54]	2	1st Maintenance	CTLA-4	Ipirimab BSC	57 57	12.7 months 12.1 months NR	2.92 months 4.9 months HR 1.44, $p = 0.097$	1.8 7

BSC best supportive care, CapeOX capecitabine plus oxaliplatin, CI confidence interval, CTLA-4 cytotoxic T-lymphocyte-associated protein 4, ECX epirubicin plus cisplatin plus capecitabine, EGFR epidermal growth factor receptor, EOC epirubicin plus oxaliplatin plus capecitabine, FOLFIRI 5-fluorouracil plus leucovorin plus oxaliplatin, FP 5-fluorouracil plus cisplatin, HER2 human epidermal growth factor receptor 2, HGF hepatocyte growth factor, HR hazard ratio, IP intraperitoneally, IV intravenously, LV leucovorin, mTOR mammalian target of rapamycin, NR not reported, ORR objective response rate, OS overall survival, PARP poly(ADP-ribose) polymerase, PFS progression-free survival, SOL S-1 plus leucovorin plus oxaliplatin, SP S-1 plus cisplatin, T-DM1 ado-trastuzumab emtansine, VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor, VEGFR2 vascular endothelial growth factor receptor 2, XP capecitabine plus cisplatin

Table 2 Ongoing phase 3 trials for advanced gastric cancer

Name of trial	Line	Control arm	Agents (experimental)	Target
JACOB	1st	XP or FP + trastuzumab	XP or FP + trastuzumab + pertuzumab	HER2
SOLAR	1st	S-1 + cisplatin	TAS-118 + oxaliplatin	Cytotoxic
JCOG1013	1st	S-1 + cisplatin	S-1 + cisplatin + docetaxel	Cytotoxic
RAINFALL	1st	XP	XP + ramucirumab	VEGFR2
KEYNOTE-062	1st	XP	XP + pembrolizumab	PD-1
JAVELIN Gastric 100	1st (maintenance)	Continuation of 1st line	Avelumab	PD-L1
ENRICH	2nd	Irinotecan	Irinotecan + nimotuzumab	EGFR
BRIGHTER	2nd	Paclitaxel	Paclitaxel + BBI608	STAT3
KEYNOTE-061	2nd	Paclitaxel	Pembrolizumab	PD-1
JAVELIN Gastric 300	3rd	Paclitaxel/irinotecan/BSC	Avelumab	PD-L1
INTEGRATE-2 (planned)	3rd or 4th	Placebo	Regorafenib	VEGFR, RET, RAF
ONO-4538-12	Last	Placebo	Nivolumab	PD-1
Tags	Last	Placebo	TAS-102	Cytotoxic

BSC best supportive care, EGFR epidermal growth factor receptor, FP 5-fluorouracil plus cisplatin, HER2 human epidermal growth factor receptor 2, PD-1 programmed cell death 1, PD-L1 programmed death ligand 1, STAT3 signal transducer and activator of transcription 3, VEGFR vascular endothelial growth factor receptor, VEGFR2 vascular endothelial growth factor receptor 2, XP capecitabine plus cisplatin

global phase 3 Tags trial is ongoing to investigate the efficacy and safety of TAS-102 in patients with AGC refractory to standard treatments (NCT02500043).

The control of peritoneal dissemination is extremely important in treatment of AGC. Intraperitoneal administration of paclitaxel provides sustained high local concentrations, and its efficacy has been shown in ovarian cancer. On the basis of previous promising results in a phase 2 trial of intraperitoneal administration of paclitaxel in combination with S-1 plus intravenous administration of paclitaxel, a phase 3 trial was conducted in Japan (PHOENIX-GC trial) [21]. A total of 183 patients were enrolled, and 164 patients were included in the efficacy analysis. The OS showed an increasing trend for intravenous regimens (median 17.7 months vs 15.2 months), although this did not reach statistical significance (Table 1). Further study might be necessary to clarify suitable candidates for intraperitoneal therapy.

Molecular targeting agents

Various drugs targeting HER2, EGFR, hepatocyte growth factor, MET, and mammalian target of rapamycin have been evaluated in AGC [22–31], but most of them did not demonstrate significant benefit in global phase 3 trials (Table 1). Even ado-trastuzumab emtansine (TDM-1), one of the antibody–drug conjugates targeting HER2 that demonstrates remarkable effectiveness in breast cancer, could not significantly prolong OS of HER2-positive AGC patients (GATSBY study) [24]. Although the exact reason for the lower efficacy in AGC than in breast cancer is not clear, it might be partially caused by the heterogeneity of

RTK expression in gastric cancers or by changes in HER2 expression after first-line treatment [32–34]. Detailed analysis of biomarkers in the GATSBY study is eagerly awaited. A phase 3 trial of pertuzumab, a monoclonal antibody for the dimerization domain of HER2, in combination of cytotoxic chemotherapy and trastuzumab therapy is ongoing (JACOB).

Large phase 3 trials of anti-EGFR monoclonal antibodies in combination with first-line chemotherapy could not show sufficient survival benefit in AGC [25, 26]. However, these two studies did not enrich patients by biomarkers. Nimotuzumab, another anti-EGFR monoclonal antibody, showed a trend of increased OS in patients with high EGFR expression [31]. A phase 3 trial in patients with EGFR-positive AGC comparing nimotuzumab plus irinotecan versus irinotecan monotherapy is ongoing (ENRICH study).

Although promising results have been obtained with MET inhibitors or FGFR inhibitors in patients with amplification of the encoding oncogenes [35–37], cases with high gene amplification rarely occur, which makes drug development challenging. A randomized phase 2 trial of AZD4547 as an FGFR inhibitor for *FGFR2*-amplified AGC did not show a superior outcome with this agent in comparison with paclitaxel [38]. This study enrolled patients who met the following criteria: polysomy (FISH4/5, ratio < 2.0 and ≥ 4 copies in 10 % or more of cells), low amplification (FISH6, ratio > 2.0), or high amplification (FISH6, ratio ≥ 5.0) according to the *FGFR2* status. However, very few patients with high amplification were treated. Exploratory biomarker analysis revealed marked intratumor heterogeneity of *FGFR2* amplification. In contrast, with a similar agent, three patients with very high *FGFR2* amplification and homogeneous expression of *FGFR2* showed a remarkable response in

another study [39]. Therefore the definition and the optimal cutoff of each biomarker is very important. Also, the treatment strategy to overcome intratumoral heterogeneity with use of optimal combinations warrants further evaluation in AGC.

The SCRUM project [39], a genome screening project in Japan as joint research with major domestic facilities and pharmaceutical companies, is under way, and enrollment for clinical studies targeting rare genomic alterations is anticipated. Patients with specific genomic alterations such as *PIK3CA* mutation, *BRAF* mutation, *AKT1* mutation, *MET* or *FGFR2* amplification, and *FGFR3–TACC3* fusion have been enrolled in early clinical trials.

Therapy with ramucirumab, a fully human IgG1 monoclonal antibody to the extracellular vascular endothelial growth factor (VEGF)-binding domain of VEGFR2, has become one of the standard chemotherapies for pretreated AGC on the basis of the findings of two pivotal phase 3 trials (Table 1) [9, 10]. Efficacy of ramucirumab was suggested to be observed in a Japanese subgroup in the RAINBOW study [40]. Although efficacy of ramucirumab was also evaluated in a randomized phase 2 study in the first-line treatment, no increase in PFS was observed [41]. A higher rate of discontinuation of study treatment for reasons other than progressive disease in the ramucirumab arm compared with the placebo arm was observed, which led to lower study drug exposure in the experimental arm. Meanwhile, exposure–efficacy response analyses performed on data obtained from REGARD and RAINBOW demonstrated that an increase in exposure is associated with improvement in efficacy in terms of both OS and PFS [42]. The phase 3 RAINFALL trial comparing PFS for an intensive dose of ramucirumab (administration on days 1 and 8 every 21 days) with cisplatin plus capecitabine (or 5-FU) versus placebo with cisplatin plus capecitabine (or 5-FU) as first-line treatment is ongoing (NCT02314117). Apatinib is a multikinase inhibitor which mainly targets VEGFR2 and has been developed in China. Apatinib significantly increased OS in patients with pretreated AGC [43]. Similarly, regorafenib, a multikinase inhibitor, significantly prolonged PFS compared with placebo as second-line or later-line therapy for AGC [44]. The OS showed an increasing trend with more than 50 % of patients in the placebo arm receiving regorafenib as cross-over treatment. A phase 3 trial is being planned.

Other drugs targeting the mechanisms of resistance to conventional chemotherapies are under development for AGC. In a preclinical study using poly(ADP-ribose) polymerase inhibitors, it was shown that these agents are effective for cell lines resistant to platinum-based agents [45]. Also, some effect has been suggested in ovarian cancer with or without BRCA mutations in clinical studies [46], leading to development in AGC patients. Promising

results were obtained in a randomized phase 2 clinical study [47] which showed significant efficacy in terms of OS (Table 1). Low expression of ataxia telangiectasia mutated (ATM) protein was suggested to be associated with remarkable efficacy. However, recently it was reported by AstraZeneca that the phase 3 GOLD trial could not meet its primary end point of improving OS in all cohorts as well as the ATM-negative cohort. Detailed reports as well as biomarker analysis are awaited.

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor located downstream of a variety of cytokines and Janus kinase, and it was reported that phosphorylated STAT3 activated the transcription of the *Nanog* and *Myc* genes, which are involved in the stemness of cells inside the nucleus, and that high expression of STAT3 was responsible for poor prognosis in multiple cancers [48]. BBI608 is a drug that specifically inhibits cancer stem-like cells that have CD44 positivity, high expression of STAT3, and the capability to form spheres [48]. Encouraging anticancer activity of BBI608 and paclitaxel in refractory AGC was observed in a phase 1b study and a subsequent phase 2 study including 46 AGC patients, with an objective response rate (ORR) of 31 % and a disease control rate of 75 % [49]. The phase 3 BRIGHTER trial comparing BBI608 plus paclitaxel and placebo plus paclitaxel for AGC patients after first-line chemotherapy is ongoing.

Immune checkpoint inhibitors

Pembrolizumab is a humanized IgG4 monoclonal antibody without antibody-dependent cell-mediated cytotoxicity, and competitively inhibits the binding of programmed cell death 1 (PD-1) to PD-L1 and PD-L2. In a phase 1b gastric clinical study (KEYNOTE-012), 39 patients (19 Asians and 20 non-Asians) with PD-L1-positive AGC received pembrolizumab [50]. Immunohistochemistry with PD-L1 antibody (22C3 antibody) was performed as screening, and those with 1 % or more staining in cancer cells or any staining of stromal cells were assessed as PD-L1 positive. Sixty-five of 162 patients (40 %) were PD-L1 positive. The ORR by the central assessment was 22.2 % and that of investigator judgment was 33 %, and a reduction in the size of the target lesions was observed in 53.1 % of the patients. Although the median PFS was 1.9 months, the 6-month survival rate was 69 %, and the median response duration was as long as 40 weeks, with an excellent median OS of 11.4 months despite the fact that 67 % of the patients had received two or more of lines of prior therapy. No significant difference in clinical outcomes was observed between Asians and non-Asians in terms of response, PFS, and OS [50]. The treatment-related adverse events were almost

similar to those of a previous study for other solid tumors. Clinical trials of pembrolizumab are currently under way in each line of therapy, including a large phase 2 trial (KEYNOTE-059; NCT02335411), a phase 3 trial (KEYNOTE-061; NCT02370498) comparing pembrolizumab with paclitaxel as second-line treatment, and a phase 3 trial (KEYNOTE-062; NCT02494583) comparing single-agent pembrolizumab or a combination therapy of 5-FU (or capecitabine) plus cisplatin plus pembrolizumab or placebo as first-line treatment for patients with PD-L1-positive and HER2-negative AGC.

Nivolumab is a humanized IgG4 recombinant anti-PD-1 monoclonal antibody. In a phase 1/2 trial (CheckMate-032), 59 patients were treated with nivolumab monotherapy, and 83 % of the patients had received two or more prior regimens [51]. The ORR was 14 %, the median PFS was 1.4 months, and the median OS was 5.0 months. The 6-month and 12-month survival rates were 49 and 36 % respectively. A phase 3 clinical study of nivolumab in AGC patients refractory to two or more lines of treatment (ONO-4538-12) has finished recruitment and the results will be reported soon. Further, in a phase 1 trial with an expansion cohort of Japanese patients with gastric cancer, the anti-PD-L1 antibody avelumab demonstrated an ORR of 15 %, with a median PFS of 11.9 weeks [52]. Two phase 3 studies of maintenance therapy after first-line therapy (JAVELIN Gastric 100; NCT02625610) and third-line therapy (JAVELIN Gastric 300; NCT02625623) are ongoing. Although there have been no established biomarkers of immune checkpoint inhibitors to date, an association has been suggested, in several different types of cancers, between therapeutic effects and PD-L1 expression, types of tumor-infiltrating lymphocytes, the number of somatic mutations (mainly passenger mutation), and immune-related gene expression in tumor tissues. At this time the definitive impact of PD-L1 expression or RNA signature as biomarkers in AGC is not clear, and will be better analyzed in a cohort of larger sample size from ongoing phase 3 trials. MSI-high tumors harbor hundreds to thousands of mutations that may produce neoantigens that can be recognized and targeted by T cells. MSI-high colorectal cancer and noncolorectal cancers, including AGC, are very sensitive to immune checkpoint inhibitors, with an ORR of around 50 % [53].

As mentioned before, single-agent response rates of immune checkpoint inhibitors are still around 10–20 %, and it is important to develop combination treatments to improve treatment outcomes. These strategies include combination of systemic chemotherapy, molecular targeting agents, radiotherapy, and local injection of oncolytic virus to enhance the local immunity and combination with immune checkpoint inhibitors such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), and T-cell immunoglobulin domain and mucin domain 3

(TIM-3), inhibition of suppressive factors such as indoleamine 2,3-dioxygenase or transforming growth factor β , and depletion of suppressive lymphocytes such as C–C motif chemokine receptor 4 (CCR4) antibody. Although monotherapy with the anti-CTLA-4 antibody ipilimumab did not show superior efficacy as maintenance therapy after first-line chemotherapy in comparison with best supportive care in a randomized phase 2 trial (Table 1) [54], combination of nivolumab and ipilimumab in the CheckMate-032 trial showed a relatively higher ORR than monotherapy (14 % with nivolumab monotherapy and 26 % with a lower dose of nivolumab in combination with ipilimumab) [55]. A phase 3 trial of this combination is being planned. Furthermore, combination of the anti-CTLA-4 antibody tremelimumab with the anti-PDL1 antibody MED4736 and single-agent MED4736 and tremelimumab therapies are also evaluated in AGC. Regarding the combination of the existing molecular targeted drugs and immune checkpoint inhibitors, a phase 1 study of the combination of ramucirumab and pembrolizumab is ongoing [56]. VEGF-A in tumor tissues is reported to induce the expression of inhibitory molecules and to exhaust CD8⁺ T cells in a mouse model, indicating the possibility of the combined use of immune checkpoint inhibitors and a VEGF inhibitor [57].

Future perspectives

Although the perspective of treatment of AGC in the near future depends on the results of ongoing clinical trials, individualized choice of treatment will become more important. Optimal timing to combine treatments and the optimal sequence of each treatment should also be evaluated in future clinical trials. Timely monitoring of tumor profiles and patients' genomic or immunological condition will be also be important to select each therapy and establish personalized therapy in AGC.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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