Updated Efficacy Outcomes of Anti-PD-1 Antibodies plus Multikinase Inhibitors for Patients with Advanced Gastric Cancer with or without Liver Metastases in Clinical Trials

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ABSTRACT

Purpose: We previously reported preliminary activity of regorafenib plus nivolumab (REGONIVO) or lenvatinib plus pembrolizumab (LENPEM) in advanced gastric cancer (AGC). Meanwhile, several studies demonstrated liver metastases are less responsive to immunotherapy.

Patients and Methods: Combined efficacy outcomes with a longer follow-up in a phase Ib trial of REGONIVO and a phase II trial of LENPEM were examined in AGC with or without liver metastases (REGONIVO plus LENPEM cohort). We also investigated the efficacy of anti-PD-1 monotherapies (anti-PD-1 monotherapy cohort). A comparison of the immune microenvironment between gastric primary tumors and liver metastases was also conducted by multiplex IHC.

Results: In the REGONIVO plus LENPEM cohort, with a median follow-up of 14.0 months, objective response rate (ORR), median progression-free survival (mPFS), and median overall survival (mOS) were 46%, 7.8 months, and 15.6 months in patients with liver metastases, while 69%, 6.9 months, and 15.5 months in those without. In the anti-PD-1 monotherapy cohort, with a median follow-up of 27.6 months, ORR, mPFS, and mOS were 9%, 1.4 months, and 6.4 months in patients with liver metastases, while 22%, 2.3 months, and 9.0 months in those without. Multiplex IHC revealed liver metastases were associated with an abundance of immune-suppressive cells, such as tumor-associated macrophages and regulatory T cells, with fewer $CD8^+$ T cells compared with gastric primary tumors.

Conclusions: Anti-PD-1 antibodies plus regorafenib or lenvatinib for AGC showed promising antitumor activity with a longer follow-up, irrespective of liver metastases status, despite a more immune-suppressive tumor microenvironment in liver metastases.

Introduction

Recently, immune checkpoint inhibitors (ICI) such as anti–programmed cell death-1 (PD-1) or programmed cell death ligand-1 (PD-L1) mAbs have become one of the standards of care for various types of cancers including advanced gastric cancer (AGC; refs. 1–8). In AGC, nivolumab, monoclonal anti-PD-1 antibody, improved survival outcomes in pivotal phase III trials such as the Asian ATTRACTION-2 study in the third-line or subsequent treatment and the global CheckMate-649 study in the first-line treatment combined with standard cytotoxic agents (8, 9). Most recently, adding pembrolizumab to trastuzumab and chemotherapy improved the response rate in the first-line treatment for patients with HER2-positive AGC in the phase III KEYNOTE-811 study (10). However, a substantial number of patients with AGC showed resistance to ICIs, highlighting the importance of the development of further combined immunotherapy.

In previous reports, inhibition of the VEGF pathway could suppress tumor growth together with the inhibition of immune-suppressive cell infiltration such as tumor-associated macrophages, regulatory T cells, and myeloid-derived suppressor cells, while increasing the mature dendritic cell fraction (11, 12). In an *in vivo* model, multikinase inhibitors of VEGF receptors and other receptor tyrosine kinases substantially decreased immune-suppressive cells with the enhancement of antitumor activity of PD-1 inhibitors (13–15). Indeed, we previously reported the promising antitumor activity of anti-PD-1 antibodies plus multikinase inhibitors [regorafenib plus nivolumab (REGONIVO) or lenvatinib plus pembrolizumab (LENPEM)] for AGC in early clinical trials (16, 17). Meanwhile, several preclinical

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Translational Relevance

We previously reported promising anti-PD-1 antibodies plus multikinase inhibitors for advanced gastric cancer (AGC). Meanwhile, several studies demonstrated liver metastases were less responsive to immune checkpoint inhibitors (ICI). We investigated updated efficacy outcomes of anti-PD-1 antibodies plus multikinase inhibitors with or without liver metastases. Impact of the presence of liver metastases on efficacy of anti-PD-1 monotherapies in AGC was also investigated. Furthermore, comparison of immune microenvironments between gastric primary tumor and liver metastases was conducted by multiplex IHC. Anti-PD-1 antibodies plus multikinase inhibitors showed promising antitumor activity with longer follow-up, irrespective of liver metastases. Meanwhile, efficacy outcomes with anti-PD-1 monotherapies were worse in patients with liver metastases than those without. Multiplex IHC revealed liver metastases was associated with an abundance of immune-suppressive cells compared with gastric primary tumor. These results suggest targeting immune-suppressive cells by multikinase inhibitors could overcome the resistance to ICIs in AGC with liver metastases.

and clinical studies demonstrated that liver metastases were less responsive to ICIs, presumably due to enriched immune-suppressive cells in liver metastases (18–22). We hypothesized that targeting immune-suppressive cells with multikinase inhibitors could overcome the resistance to ICIs in patients with AGC with liver metastases.

Here, we investigated updated efficacy outcomes of REGONIVO and LENPEM for AGC with or without liver metastases in these clinical trials. The impact of the presence of liver metastases on the efficacy of anti-PD-1 monotherapies in AGC was also investigated. Furthermore, comparison of the immune microenvironments between gastric primary tumors and liver metastases was conducted by multiplex IHC.

Patients and Methods

Patients

The current study examined combined efficacy outcomes with a longer follow-up in a phase Ib trial of REGONIVO (NCT 03406871) and a phase II trial of LENPEM (NCT 03609359) in patients with AGC (16, 17). The detailed methods of these trials were reported previously (16, 17). We also retrospectively reviewed the medical records of patients with AGC treated with anti-PD-1 monotherapy at the National Cancer Center Hospital East. The eligibility criteria for the anti-PD-1 monotherapy cohort were as follows: (i) an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; (ii) histologically proven, unresectable, locally advanced or metastatic gastric adenocarcinoma; (iii) adequate bone marrow, hepatic, and renal function; and (iv) history of previous treatment with one or more regimens and at least one treatment with nivolumab or pembrolizumab from September 2017 to September 2019. Efficacy outcomes were compared between patients with liver metastases and those without liver metastases in the REGONIVO plus LENPEM cohort and the anti-PD-1 monotherapy cohort. We also additionally analyzed survival outcomes according to the presence of peritoneum metastases.

All the patients provided written informed consent prior to chemotherapy. Furthermore, patients who underwent biomarker analysis provided written informed consent for the analysis. The study protocol was approved by the Institutional Review Board at the National Cancer Center Japan. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Molecular characteristics

As reported previously, molecular characteristics, such as the status of HER2, PD-L1, and mismatch repair deficiency (MMR) were analyzed with formalin-fixed paraffin-embedded (FFPE) tissue specimens from archival tissue samples if available (23, 24). IHC using a monoclonal anti-HER2 antibody [PATHWAY HER2 (4B5)] and FISH using the PathVysion HER-2 probe kit (Abbott Laboratories) were performed to assess HER2 status, and HER2 positive was defined as IHC 3 + or IHC 2 + and FISH positive. PD-L1 IHC was performed using an anti-PD-L1 mAb (Clone 28-8, 22C3, SP142, or SP263) and measured using a combined positive score (CPS), defined as the number of PD-L1–positive cells (tumor cells, lymphocytes, and macrophages) as a proportion of the total number of tumor cells multiplied by 100. MMR status was assessed by IHC using mAbs for anti-mutL homolog 1 (MLH1, ES05), anti-mutS homolog 2 (MSH2, FE11), anti-postmeiotic segregation increased 2 (PMS2, EP51), and anti-mutS homolog 6 (MSH6, EP49; Agilent Technologies), and tumors lacking either MLH1, MSH2, PMS2, or MSH6 expression were considered MMR deficient, whereas tumors that maintained expression of MLH1, MSH2, PMS2, and MSH6 were considered MMR proficient. All specimens in this study were reviewed by a pathologist (T. Kuwata).

Outcomes and statistical analysis

We assessed the objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) for each cohort. Tumor response was assessed in patients with measurable lesions according to the guidelines of the RECIST version 1.1. The ORR was defined as the proportion of patients with the best overall response of complete response (CR) or partial response (PR). The DCR was defined as the proportion of patients with the best overall response of CR, PR, or stable disease (SD). The PFS was defined as the time from the patient enrollment (REGONIVO or LENPEM) or the initiation of nivolumab or pembrolizumab (anti-PD-1 monotherapy cohort) until the date of disease progression or the date of death from any cause. The OS was defined as the time from the patient enrollment (REGONIVO or LENPEM) or the initiation of anti-PD-1 monotherapy until the date of death from any cause. Statistical comparison of the ORR and DCR according to the presence of liver metastases was performed using Fisher exact test. The PFS and OS were estimated by the Kaplan–Meier method, compared according to the presence of liver metastases using Cox proportional hazards models, and presented as HRs with 95% confidence intervals (CI). PFS and OS in anti-PD-1 monotherapy cohort were analyzed using multivariate Cox regression analyses. Confounders in multivariate analysis included age (≥65 vs. <65), sex (male vs. female), ECOG PS (0 vs. 1), histology (intestinal vs. diffuse), numbers of previous chemotherapy (2 vs. ≥3), metastatic site (lymph node, liver, lung, and peritoneum), HER2 status (positive vs. negative), MMR (deficient vs. proficient), and CPS (≥10 vs. <10). Statistical analyses were done using SAS software (version 9.4).

Multiplex fluorescent IHC

Next, we performed multiplex IHC (mIHC) to compare the immune microenvironments between gastric primary tumors and

Table 1. Baseline characteristics.

		REGONIVO plus LENPEM cohort ($n = 54$)	Anti-PD-1 monotherapy cohort ($n = 136$)
Age	Median (range)	68 (40–83)	68 (33–86)
	<65	20 (54%)	46 (34%)
	≥65	34 (63%)	90 (66%)
Gender	Male	48 (89%)	98 (72%)
	Female	6 (11%)	38 (28%)
ECOG PS	0	50 (93%)	71 (52%)
	≥1	4 (7%)	65 (48%)
Histology	Intestinal	29 (54%)	62 (52%)
	Diffuse	25 (46%)	74 (54%)
Number of previous chemotherapy	0	14 (26%)	0
	1	15 (28%)	4 (3%)
	2	9 (17%)	65 (48%)
	≥3	16 (30%)	67 (49%)
Site of metastases	Lymph node	46 (85%)	101 (74%)
	Liver	28 (52%)	46 (34%)
	Lung	9 (17%)	21 (15%)
	Peritoneum	15 (28%)	85 (63%)
HER2	Positive	11 (20%)	22 (16%)
	Negative	43 (80%)	108 (79%)
	Missing	0	6 (4%)
EBV	Positive	2 (4%)	5 (4%)
	Negative	52 (96%)	116 (85%)
	Missing	0	15 (11%)
MMR	Deficient	2 (4%)	14 (10%)
	Proficient	52 (96%)	109 (80%)
	Missing	0	13 (10%)
PD-L1 CPS	<1	24 (44%)	24 (18%)
	≥1	29 (54%)	92 (68%)
	≥10	6 (11%)	35 (26%)
	Missing	1 (2%)	20 (15%)

Abbreviations: CPS, combined positive score; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2; LENPEM, lenvatinib plus pembrolizumab; MMR, mismatch repair; REGONIVO, regorafenib plus nivolumab.

liver metastases using biopsy or surgical tumor samples in patients with AGC. Gastric primary tumor and liver metastases were obtained from the same patients without prior chemotherapy at the National Cancer Center Hospital East, from January 2009 to May 2019. The FFPE blocks of tumor samples were sliced into 4-μm-thick sections onto adhesion microscope slides (Matsunami). The tissue slides were deparaffinized and rehydrated for mIHC staining. Antigen retrieval and staining followed the protocol of Opal 7-Color IHC Kits (AKOYA Biosciences) provided by the manufacturer. Images were acquired using a Vectra 3 System (PerkinElmer). The protein expression levels of CD4 (Clone 4B12), CD8α (Clone C8/144B), CD206 (Clone CL0387), CD11b (Clone D6×1N), FOXP3 (Clone 236A/E7), and cytokeratin (Clone AE1/AE3) were assessed. Cell phenotyping was identified by inForm Tissue Analysis Software (AKOYA Biosciences), and cell density was calculated from the average density of at least three regions of interest (682 μm × 510 μm/region). The density of the indicated immune cells was plotted using a heatmap and was normalized by Z-score transformation. The feature of infiltrating immune cells was analyzed by principal component analysis, and the differences between organs were analyzed by an analysis of similarities (ANOSIM).

Data availability

The data generated in this study are available upon request from the corresponding authors.

Results

Patient characteristics

Baseline patient characteristics for each cohort are shown in **Table 1**. Each characteristic of REGONIVO and LENPEM is also available in Supplementary Table S1. Most patients had an ECOG PS of 0 in the REGONIVO plus LENPEM cohort, while about half of the patients had an ECOG PS of 0 in the anti-PD-1 monotherapy cohort. Liver metastases were observed in 28 (52%) and 46 (34%) patients in the REGONIVO plus LENPEM cohort and anti-PD-1 monotherapy cohort, respectively. Peritoneum metastases were frequently observed in the anti-PD-1 monotherapy cohort compared with the REGONIVO plus LENPEM cohort: 85 (63%) and 15 (28%) patients, respectively.

Efficacy

REGONIVO plus LENPEM cohort

The data cutoff for the updated efficacy analysis was December 15, 2020, with a median follow-up of 14.0 months (range, 2.0–31.3 months). All patients ($n = 54$) had measurable lesions. The ORR and DCR were 57% (31/54 patients) and 94% (51/54 patients) in the overall population (**Table 2**). The ORR was 46% in patients with liver metastases and 69% in patients without liver metastases ($P = 0.0938$; **Table 2**; **Fig. 1A**). The median PFS was 7.0 months (95% CI, 5.4–9.7) in the overall population (**Fig. 1B**). The median PFS was 7.8 months (95% CI, 4.3–13.7) with liver metastases and 6.9 months

Table 2. Tumor response.

	REGONIVO plus LENPEM cohort				Anti-PD-1 monotherapy cohort			
	All	Liver metastases		P^a	All	Liver metastases		P^a
		−	+			−	+	
Number	54	26	28		136	90	46	
Measurable lesion+	54	26	28		109	64	45	
CR	2	1	1		2	2	0	
PR	29	17	12		16	12	4	
SD	20	6	14		22	15	7	
PD	3	2	1		67	34	33	
NE	0	0	0		2	1	1	
ORR (%)	57%	69%	46%	0.0938	17%	22%	9%	0.1144
DCR (%)	94%	92%	96%	0.5187	37%	45%	24%	0.0285

Abbreviations: CR, complete response; DCR, disease control rate; LENPEM, lenvatinib plus pembrolizumab; NE, not evaluated; ORR, objective response rate; PD, progressive disease; PR, partial response; REGONIVO, regorafenib plus nivolumab; SD, stable disease.
[a]Fisher exact test was used to compare ORR and DCR.

(95% CI, 4.6–9.8) without liver metastases [HR: 0.817 (95% CI, 0.462–1.444), $P = 0.4813$; **Fig. 1C**]. The median OS was 15.6 months (95% CI, 10.6–24.5) in the overall population, with 35 patients (65%) being already deceased (**Fig. 1D**). The median OS was 15.6 months (95% CI, 9.8–not reached) with liver metastases and 15.5 months (95% CI, 7.2–22.2) without liver metastases [HR: 0.723 (95% CI, 0.371–1.411), $P = 0.3398$; **Fig. 1E**]. Patients with peritoneum metastases had shorter PFS and OS compared to those without (Supplementary Fig. S1A and S1B). Clinical outcomes according to HER2, MMR, and CPS were available in Supplementary Table S2. Each efficacy of REGONIVO and LENPEM is also available in Supplementary Table S3 and Supplementary Fig. S2 and S3.

Anti-PD-1 monotherapy cohort

The data cutoff was September 30, 2020, with a median follow-up of 27.6 months (range, 0.7–57.3 months). Of 136 patients, 109 patients (80%) had measurable lesions. The ORR and DCR were 17% (18/109 patients) and 37% (40/109 patients) in the overall population (**Table 2**). The ORR tended to be lower in patients with liver metastases than in those without liver metastases (9% vs. 22%, $P = 0.1144$; **Table 2**; **Fig. 2A**). The median PFS was 1.9 months (95% CI, 1.6–2.3) in the overall population (**Fig. 2B**). The PFS was significantly shorter in patients with liver metastases than in those without liver metastases [median 1.4 months (95% CI, 0.9–1.8) vs. 2.3 months (95% CI, 1.9–3.7), HR: 1.856 (95% CI, 1.276–2.698), $P = 0.0009$; **Fig. 2C**]. The median OS was 8.7 months (95% CI, 6.0–10.3) in the overall population, with 102 patients (75%) being already deceased (**Fig. 2D**). The median OS was 6.4 months (95% CI, 3.8–10.9) with liver metastases and 9.0 months (95% CI, 6.7–10.9) without liver metastases [HR: 1.253 (95% CI, 0.836–1.878), $P = 0.2724$; **Fig. 2E**]. Multivariate analysis showed that patients with liver metastases had a significantly shorter PFS (HR, 2.015; 95% CI, 1.253–3.241; $P = 0.0039$) and tended to have a shorter OS (HR, 1.662; 95% CI, 0.961–2.872; $P = 0.069$) compared with those without liver metastases. Patients with peritoneum metastases had shorter PFS and OS compared with those without (Supplementary Fig. S1C and S1D).

Comparison of the immune microenvironment between gastric primary tumors and liver metastases

Ten paired specimens of gastric primary tumors and liver metastases were analyzed by mIHC (**Fig. 3A** and **B**). The characteristics of the tumor-infiltrating immune cells showed a clear difference between gastric primary tumors and liver metastases (**Fig. 3C**). Enrichment of $CD206^+CD11b^+$ cells, which are regarded as tumor-associated macrophages, was observed in liver metastases. Principal component analysis summarized the features of the infiltrating immune cells, and an ANOSIM showed a significant difference between gastric primary tumors and liver metastases (**Fig. 3D**). The density of $CD206^+CD11b^+$ cells was significantly higher in liver metastases than in gastric primary tumors, while the density of $CD8^+$ T cells tended to be lower in liver metastases than in gastric primary tumors (**Fig. 3E**). The density of $FOXP3^+CD4^+$ cells, which are regarded as regulatory T cells, was numerically higher in liver metastases compared with gastric primary tumors, although the difference was not statistically significant. The $CD206^+CD11b^+/CD8^+$ and $FOXP3^+CD4^+/CD8^+$ ratios were significantly higher in liver metastases than in gastric primary tumors.

Discussion

In this study, we investigated the updated clinical activity of REGONIVO or LENPEM for patients with AGC in clinical trials. To the best of our knowledge, this is the first report to provide information on the efficacy of anti-PD-1 antibodies plus multikinase inhibitors with a longer follow-up for patients with AGC with or without liver metastases. Also, multiplex fluorescent IHC demonstrated the immune-suppressive microenvironment in liver metastases compared with gastric primary tumors.

In REGONIVO, with a median follow-up of 14.0 months, the median PFS and OS were 5.6 and 12.3 months in the third- or later-line setting. In LENPEM, with a median follow-up of 17.8 months, the median PFS and OS were 7.1 and 24.5 months in the first- or second-line setting. These survival outcomes seem to be better than those of standard chemotherapy in comparable treatment lines, although cross-trial comparison require careful interpretation because these are early clinical trials with a select patient population (8, 9, 25, 26). Meanwhile, REGONIVO for colorectal cancer was not as effective in North American population as in Japanese patients in REGONIVO, especially in patients with liver metastases (16, 27). Furthermore, a subgroup analysis of the phase III KEYNOTE-062 trial showed that the hazard ratio for OS (pembrolizumab to chemotherapy) was better in an Asian population than in a non-Asian population (28). Therefore, a further study would be needed to investigate the efficacy of REGONIVO or LENPEM for non-Japanese AGC patients. Currently, a phase III trial (INTEGRATEIIb; NCT 04879368) of

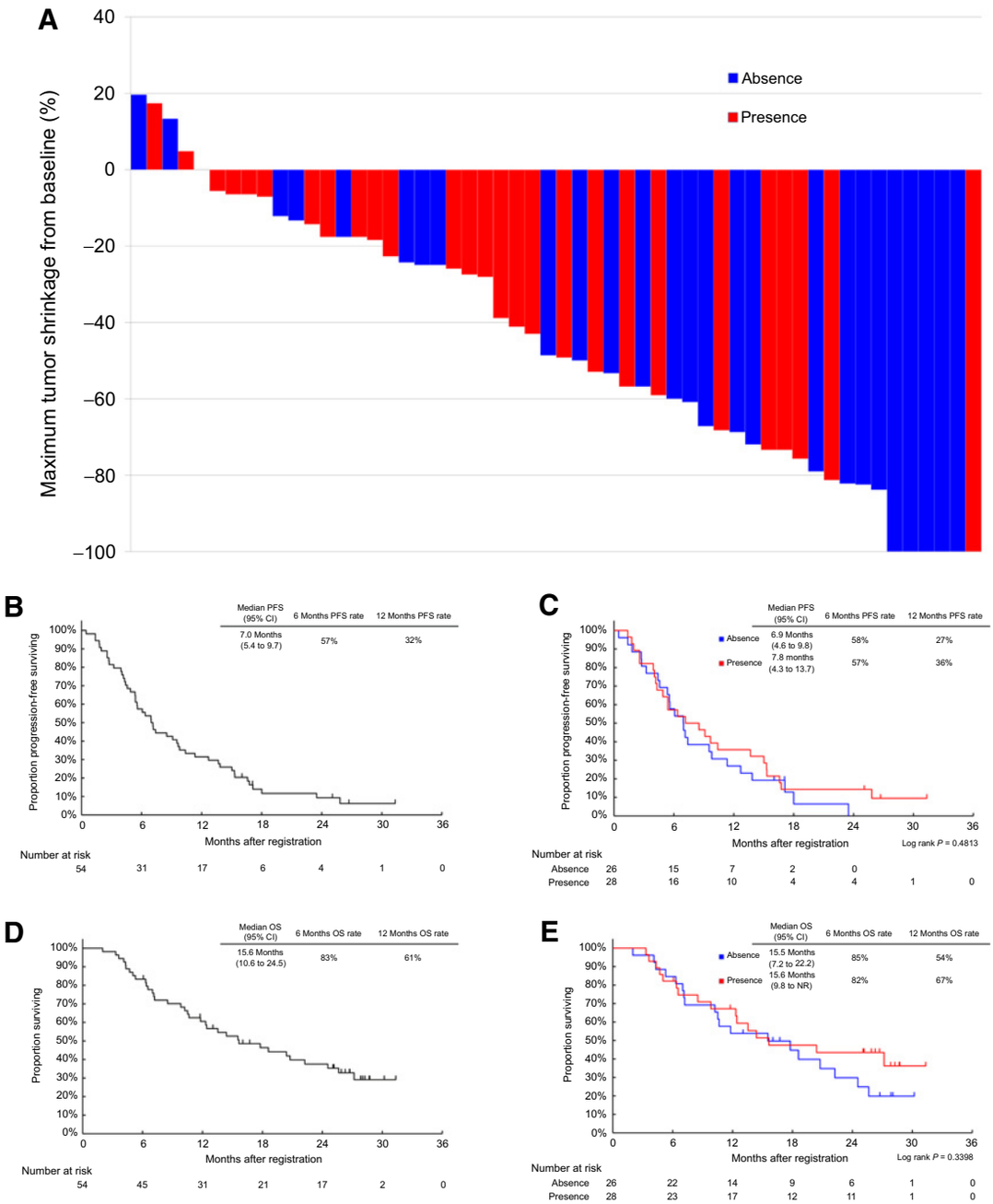

Figure 1.

Efficacy in the REGONIVO plus LENPEM cohort. **A,** Waterfall plot of maximum percent change in tumor size from baseline as measured by RECIST with or without liver metastases. **B,** Kaplan–Meier plots of PFS in the overall population. **C,** Kaplan–Meier plots of PFS according to the presence of liver metastases. **D,** Kaplan–Meier plots of OS in the overall population. **E,** Kaplan–Meier plots of OS according to the presence of liver metastases. NR, not reached; OS, overall survival; PFS, progression-free survival.

REGONIVO compared with standard chemotherapy for AGC in the third- or later-line setting and a phase III trial (LEAP-015; NCT 04662710) of LENPEM plus chemotherapy followed by LENPEM versus chemotherapy for AGC in the first-line setting is being investigated.

In this study, both REGONIVO and LENPEM showed promising clinical activity irrespective of liver metastases status. In patients with liver metastases, the ORR was 40% in REGONIVO and 54% in LENPEM with favorable survival outcomes. Meanwhile, in the anti-PD-1 monotherapy cohort, the ORR (9% vs. 22%) and PFS (1.4 vs. 2.3 months) were worse in patients with liver metastases than those without liver metastases, which is in line with previous reports showing that ICIs are less effective in patients with melanoma or non–small cell

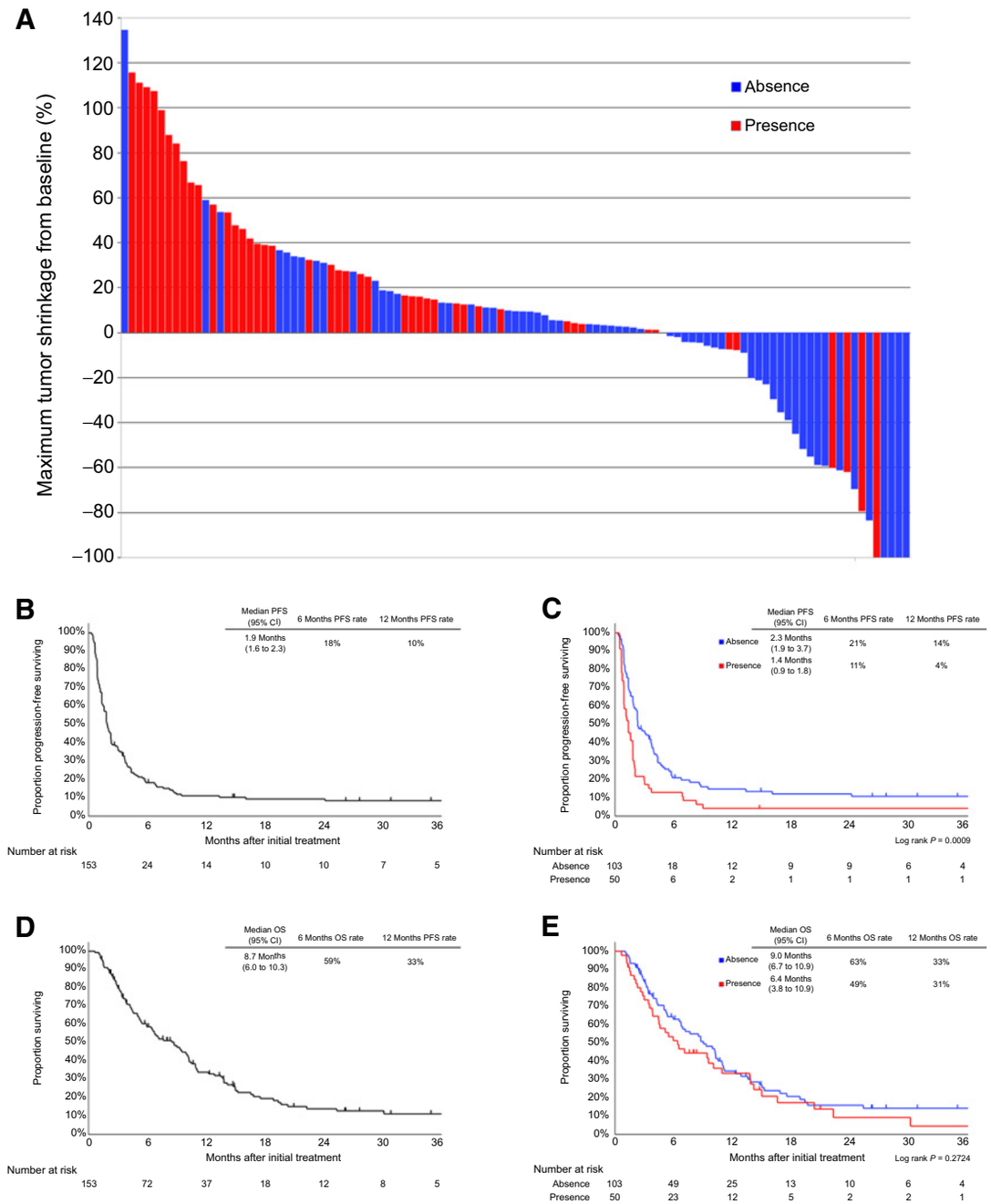

Figure 2.

Efficacy in anti-PD-1 monotherapy cohort. **A,** Waterfall plot of maximum percent change in tumor size from baseline as measured by RECIST with or without liver metastases. **B,** Kaplan–Meier plots of PFS in the overall population. **C,** Kaplan–Meier plots of PFS according to the presence of liver metastases. **D,** Kaplan–Meier plots of OS in the overall population. **E,** Kaplan–Meier plots of OS according to the presence of liver metastases. OS, overall survival; PFS, progression-free survival.

lung cancer with liver metastases (20, 21). Although the survival benefit of nivolumab was observed in the ATTRACTION-2 trial (nivolumab vs. placebo) or the ATTRACTION-4 trial (nivolumab plus chemotherapy vs. placebo plus chemotherapy) regardless of liver metastases, retrospective studies suggest that the presence of liver metastases in AGC is associated with rapid disease progression or a lower response rate compared with other metastases (8, 29–31). Recently, it was reported that liver metastases create a systemic immune desert through an interaction between T cells and macrophages in preclinical models (19). Another preclinical study also demonstrated that antigen-specific immune suppression by activation of regulatory T cells and $CD11b^{+}$ monocytes in liver metastases led to

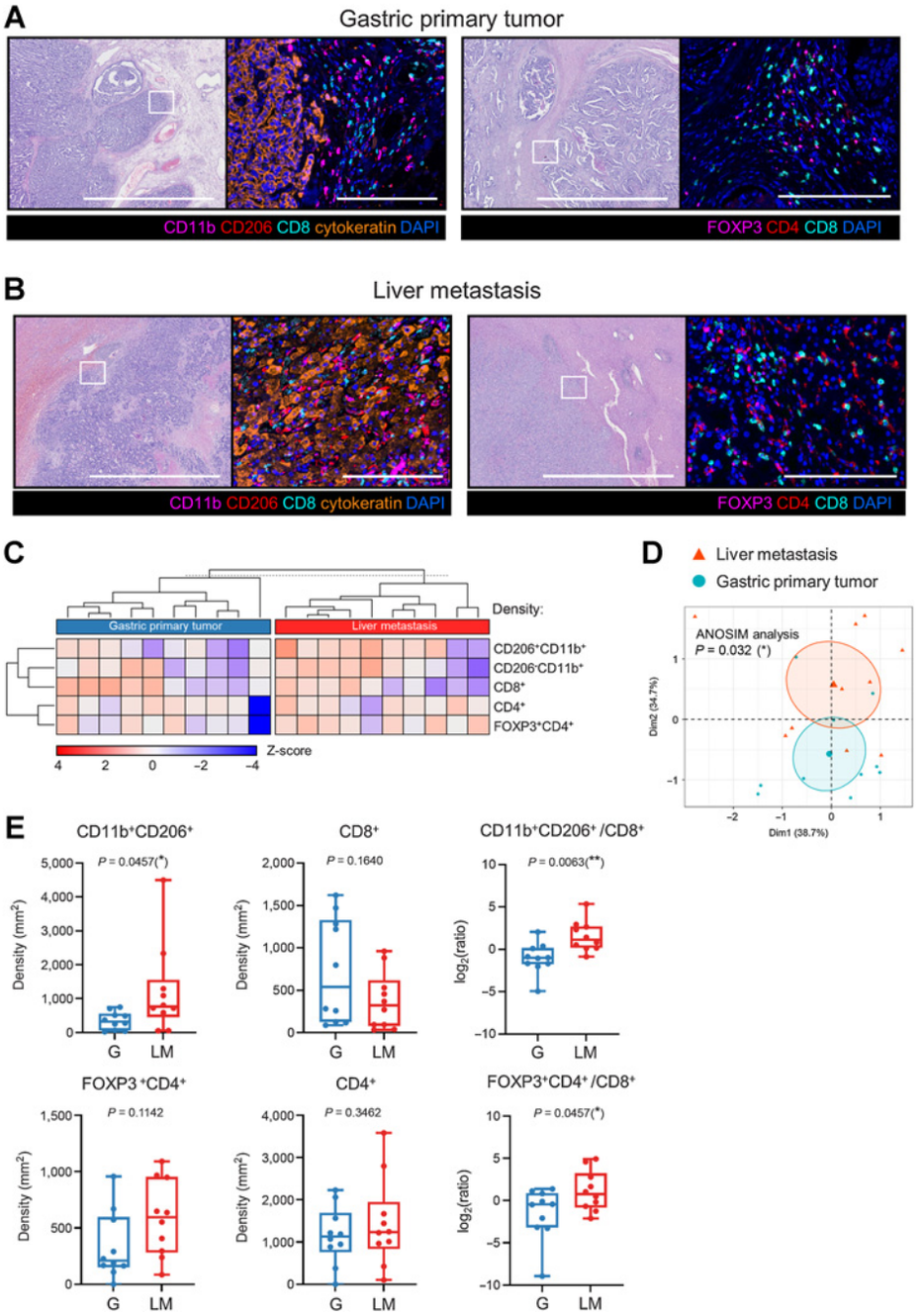

Figure 3.

Representative multiplex IHC images of gastric primary tumor (**A**) and liver metastasis (**B**). **C,** Characteristics of tumor-infiltrating immune cells from gastric primary tumor and liver metastasis. **D,** Principal component analysis and ANOSIM analysis of tumor-infiltrating immune cells from gastric primary tumor or liver metastasis. **E,** Comparative analysis of tumor-infiltrating immune cells from gastric primary tumor (G) or liver metastasis (LM).

the systemic suppression of antitumor immunity in mouse models (18). In our study, liver metastases were associated with an abundance of immune-suppressive cells, such as tumor-associated macrophages and regulatory T cells with fewer $CD8^+$ T cells, compared with gastric primary tumors. These findings from our study and previous reports suggest that targeting immune-suppressive cells by multikinase inhibitors could relieve the systemic immunosuppressive effect led by liver metastases. Also, other agents targeting immune-suppressive cells such as PGE2-receptor EP4 antagonists, TGFβ inhibitors, FLOUNT (a regulator of CCR2 and CCR5 signaling) inhibitors, and local therapies including photodynamic therapy are expected to overcome resistance to anti-PD-1 antibodies in patients with liver metastases in future studies. Meanwhile, patients with peritoneum metastases had shorter PFS and OS compared with those without in both REGONIVO plus LENPEM cohort and anti-PD-1 monotherapy cohort, in line with a previous report (32). Tumor immune microenvironment in peritoneum should also be investigated in future study.

The major limitation to the current study was the small sample size, which comprised a select population as early clinical trials. Thus, any efficacy analysis is preliminary in nature. Also, efficacy in the anti-PD-1 monotherapy cohort was investigated with a retrospective

manner at a single institution. Finally, comparison of the immune microenvironments between gastric primary tumors and liver metastases by multiple immunofluorescence IHC was performed in a limited number of tumor samples.

In conclusion, the combination of anti-PD-1 antibodies plus multikinase inhibitors (REGONIVO or LENPEM) for patients with AGC showed promising antitumor activity with a longer follow-up in these clinical trials, irrespective of liver metastases. Also, an immune-suppressive microenvironment was observed by multiple immunofluorescence IHC in liver metastases compared with gastric primary tumors. These results suggest that targeting immune-suppressive cells by multikinase inhibitors could overcome the resistance to ICIs in patients with AGC with liver metastases, which should also be investigated in ongoing pivotal phase III trials of these combination immunotherapies.

Authors' Disclosures

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Note

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References

1. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017;376:1015–26.
2. Powles T, Durán I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2018;391:748–57.
3. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018–28.
4. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. N Engl J Med 2020;383:1328–39.
5. Motzer RJ, Escudier B, George S, Hammers HJ, Srinivas S, Tykodi SS, et al. Nivolumab versus everolimus in patients with advanced renal cell carcinoma: updated results with long-term follow-up of the randomized, open-label, phase 3 checkmate 025 trial. Cancer 2020;126:4156–67.
6. Kato K, Cho BC, Takahashi M, Okada M, Lin CY, Chin K, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy

(ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2019;20:1506–17.
7. Takei S, Kawazoe A, Shitara K. The new era of immunotherapy in gastric cancer. Cancers 2022;14:1054.
8. Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538–12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461–71.
9. Janjigian YY, Shitara K, Moehler M, Garrido M, Salman P, Shen L, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. Lancet 2021; 398:27–40.
10. Janjigian YY, Kawazoe A, Yañez P, Li N, Lonardi S, Kolesnik O, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature 2021;600:727–30.
11. Tada Y, Togashi Y, Kotani D, Kuwata T, Sato E, Kawazoe A, et al. Targeting VEGFR2 with ramucirumab strongly impacts effector/activated regulatory T cells and CD8(+) T cells in the tumor microenvironment. J Immunother Cancer 2018;6:106.
12. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. Front Immunol 2018;9:978.
13. Ou DL, Chen CW, Hsu CL, Chung CH, Feng ZR, Lee BS, et al. Regorafenib enhances antitumor immunity via inhibition of p38 kinase/Creb1/Klf4 axis in tumor-associated macrophages. J Immunother Cancer 2021;9:e001657.
14. Doleschel D, Hoff S, Koletnik S, Rix A, Zopf D, Kiessling F, et al. Regorafenib enhances anti-PD1 immunotherapy efficacy in murine colorectal cancers and their combination prevents tumor regrowth. J Exp Clin Cancer Res 2021;40:288.
15. Kato Y, Tabata K, Kimura T, Yachie-Kinoshita A, Ozawa Y, Yamada K, et al. Lenvatinib plus anti-PD-1 antibody combination treatment activates CD8+ T cells through reduction of tumor-associated macrophage and activation of the interferon pathway. PLoS One 2019;14:e0212513.
16. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, et al. Regorafenib plus nivolumab in patients with advanced gastric or colorectal cancer: an open-label, dose-escalation, and dose-expansion phase Ib trial (REGONIVO, EPOC1603). J Clin Oncol 2020;38:2053–61.
17. Kawazoe A, Fukuoka S, Nakamura Y, Kuboki Y, Wakabayashi M, Nomura S, et al. Lenvatinib plus pembrolizumab in patients with advanced gastric cancer in the first-line or second-line setting (EPOC1706): an open-label, single-arm, phase 2 trial. Lancet Oncol 2020;21:1057–65.
18. Lee JC, Mehdizadeh S, Smith J, Young A, Mufazalov IA, Mowery CT, et al. Regulatory T cell control of systemic immunity and immunotherapy response in liver metastasis. Sci Immunol 2020;5:eaba0759.
19. Yu J, Green MD, Li S, Sun Y, Journey SN, Choi JE, et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. Nat Med 2021;27:152–64.
20. Tumeh PC, Hellmann MD, Hamid O, Tsai KK, Loo KL, Gubens MA, et al. Liver metastasis and treatment outcome with anti-PD-1 monoclonal antibody in patients with melanoma and NSCLC. Cancer Immunol Res 2017;5:417–24.
21. Pires da Silva I, Lo S, Quek C, Gonzalez M, Carlino MS, Long GV, et al. Site-specific response patterns, pseudoprogression, and acquired resistance in patients with melanoma treated with ipilimumab combined with anti-PD-1 therapy. Cancer 2020;126:86–97.
22. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin Y-T, Togashi Y, et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. Cancer Cell 2022;40:201–18.
23. Mishima S, Kawazoe A, Nakamura Y, Sasaki A, Kotani D, Kuboki Y, et al. Clinicopathological and molecular features of responders to nivolumab for patients with advanced gastric cancer. J Immunother Cancer 2019;7:24.
24. Ishii T, Kawazoe A, Sasaki A, Mishima S, Kentaro S, Nakamura Y, et al. Clinical and molecular factors for selection of nivolumab or irinotecan as third-line treatment for advanced gastric cancer. Ther Adv Med Oncol 2020;12: 1758835920942377.
25. Shitara K, Özgüroğlu M, Bang YJ, Di Bartolomeo M, Mandalà M, Ryu MH, et al. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. Lancet 2018;392:123–33.
26. Shitara K, Doi T, Dvorkin M, Mansoor W, Arkenau HT, Prokharau A, et al. Trifluridine/tipiracil versus placebo in patients with heavily pretreated metastatic gastric cancer (TAGS): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2018;19:1437–48.
27. Fakih M, Raghav KPS, Chang DZ, Bendell JC, Larson T, Cohn AL, et al. Single-arm, phase 2 study of regorafenib plus nivolumab in patients with mismatch repair-proficient (pMMR)/microsatellite stable (MSS) colorectal cancer (CRC). J Clin Oncol 39:15s, 2021 (suppl; abstr 3560).
28. Shitara K, Van Cutsem E, Bang YJ, Fuchs C, Wyrwicz L, Lee KW, et al. Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: the KEYNOTE-062 phase 3 randomized clinical trial. JAMA Oncol 2020;6:1571–80.
29. Sasaki A, Nakamura Y, Mishima S, Kawazoe A, Kuboki Y, Bando H, et al. Predictive factors for hyperprogressive disease during nivolumab as anti-PD1 treatment in patients with advanced gastric cancer. Gastric Cancer 2019;22: 793–802.
30. Motoo I, Ando T, Kajiura S, Ogawa K, Tsukada K, Ueda A, et al. Lesion-level response to immune checkpoint inhibitor in patients with advanced gastric cancer. J Clin Oncol 40:4s, 2022 (suppl; abstr 311).
31. Kang YK, Chen LT, Ryu MH, Oh DY, Oh SC, Chung HC, et al. Nivolumab plus chemotherapy versus placebo plus chemotherapy in patients with HER2-negative, untreated, unresectable advanced or recurrent gastric or gastro-oesophageal junction cancer (ATTRACTION-4): a randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2022;23:234–47.
32. Fucà G, Cohen R, Lonardi S, Shitara K, Elez ME, Fakih M, et al. Ascites and resistance to immune checkpoint inhibition in dMMR/MSI-H metastatic colorectal and gastric cancers. J Immunother Cancer 2022;10:e004001.