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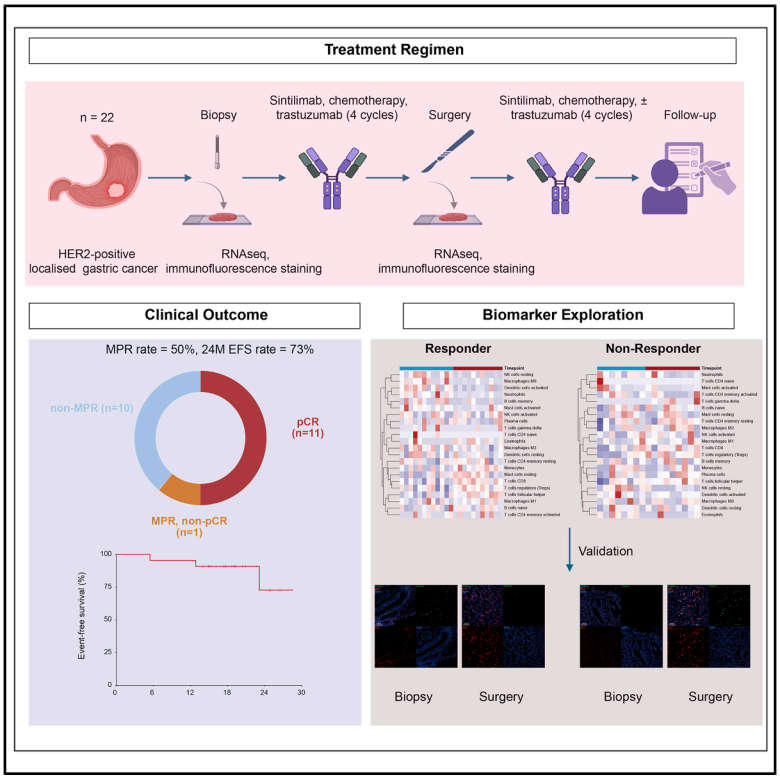
Safety and efficacy of perioperative dual PD-1 and HER2 blockade in HER2-positive gastric cancer

Graphical abstract



Highlights

- Perioperative dual PD-1 and HER2 blockade are feasible in HER2-positive gastric cancer
- The combination regimen is well tolerated and associated with promising survival
- Regulatory T cells were associated with the possibility of drug resistance

Authors

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In brief

In this phase 2 trial, Nie et al. demonstrate that perioperative sintilimab, trastuzumab, and chemotherapy have promising antitumor activity with a manageable safety profile and might be promising options for patients with localized HER2-positive gastric and gastro-esophageal junction adenocarcinoma.

Nie et al., 2025, Cell Reports Medicine 6, 102190
June 17, 2025
https://doi.org/10.1016/j.xcrm.2025.102190

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Safety and efficacy of perioperative dual PD-1 and HER2 blockade in HER2-positive gastric cancer

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https://doi.org/10.1016/j.xcrm.2025.102190

SUMMARY

The use of trastuzumab and programmed death-1 (PD-1) inhibitor is effective in patients with HER2-positive advanced gastric or gastro-esophageal junction cancer; however, their use has not been investigated in patients with localized disease. This phase 2 trial evaluates the safety and efficacy of dual PD-1 (sintilimab) and HER2 blockade with chemotherapy in patients with resectable HER2-positive gastric and gastro-esophageal junction adenocarcinoma. 22 patients are enrolled, and 20 patients undergo surgery. The primary endpoint is achieved; 12 (55%, 95% confidence interval [CI]: 32–76) of 22 patients have a major pathological response, and 11 (50%, 95% CI: 28–72) of 22 patients achieve pathological complete response. The most common grade 3 treatment-related adverse events are neutropenia and thrombocytopenia. No treatment-related deaths occur. Transcriptomic analysis, bioinformatics analysis, and immunofluorescence staining demonstrate that regulatory T cells are associated with possibility of drug resistance. This study was registered at the Chinese Clinical Trial Registry (identifier: ChiCTR2200058732).

INTRODUCTION

Gastric and gastro-esophageal junction adenocarcinoma rank as the fifth disease worldwide in terms of both incidence and mortality, with an estimated 0.97 million new diagnoses and 0.66 million deaths in 2022.[1] Patients with gastric and gastro-esophageal junction adenocarcinoma are often diagnosed in a locally advanced stage.[2] Despite curative resection being the gold standard treatment, patients with localized disease continue to have a high risk of recurrence.[3,4] While a multimodal approach integrating curative resection and perioperative chemotherapy has led to enhanced survival, the 5-year overall survival rate remains <50%,[5,6] highlighting the urgent need for more effective treatment strategies in these patients.

Approximately 17%–20% of patients with gastric and gastro-esophageal junction adenocarcinoma harbor HER2 (also known as ERBB2) amplification or protein overexpression.[7,8] The ToGA trial showed that patients exhibiting HER2 amplification or overexpression benefit from treatment with the anti-HER2 antibody trastuzumab with cytotoxic chemotherapy.[9] Furthermore, the KEYNOTE-811 trial revealed that the addition of the anti-programmed death-1 (PD-1) antibody pembrolizumab to trastuzumab and chemotherapy improved the objective response, progression-free survival, and overall survival in patients with metastatic HER2-positive gastric and gastro-esophageal junction adenocarcinoma, especially in patients with tumors with a PD-L1 combined positive score (CPS) of 1 or more.[10–12] Therefore, this regimen is recommended as a first-line therapy.

Several randomized controlled trials, including KEYNOTE-585,[13] MATTERHORN,[14] and NEOSUMMIT-01,[15] have indicated that perioperative chemotherapy with PD-1 or PD-L1 inhibitors significantly enhances the pathological response, irrespective of the HER2 status. The HER-FLOT and NEOHX trials assessed the addition of perioperative trastuzumab to chemotherapy in patients with HER2-positive localized gastric and gastro-esophageal junction adenocarcinoma, with moderate improved pathological response.[16,17] Herein, we report the results of the prospective, non-randomized, open-label, phase 2 NEOSUMMIT-02 trial to evaluate the efficacy and safety of the perioperative anti-PD-1 antibody sintilimab, trastuzumab, and chemotherapy in

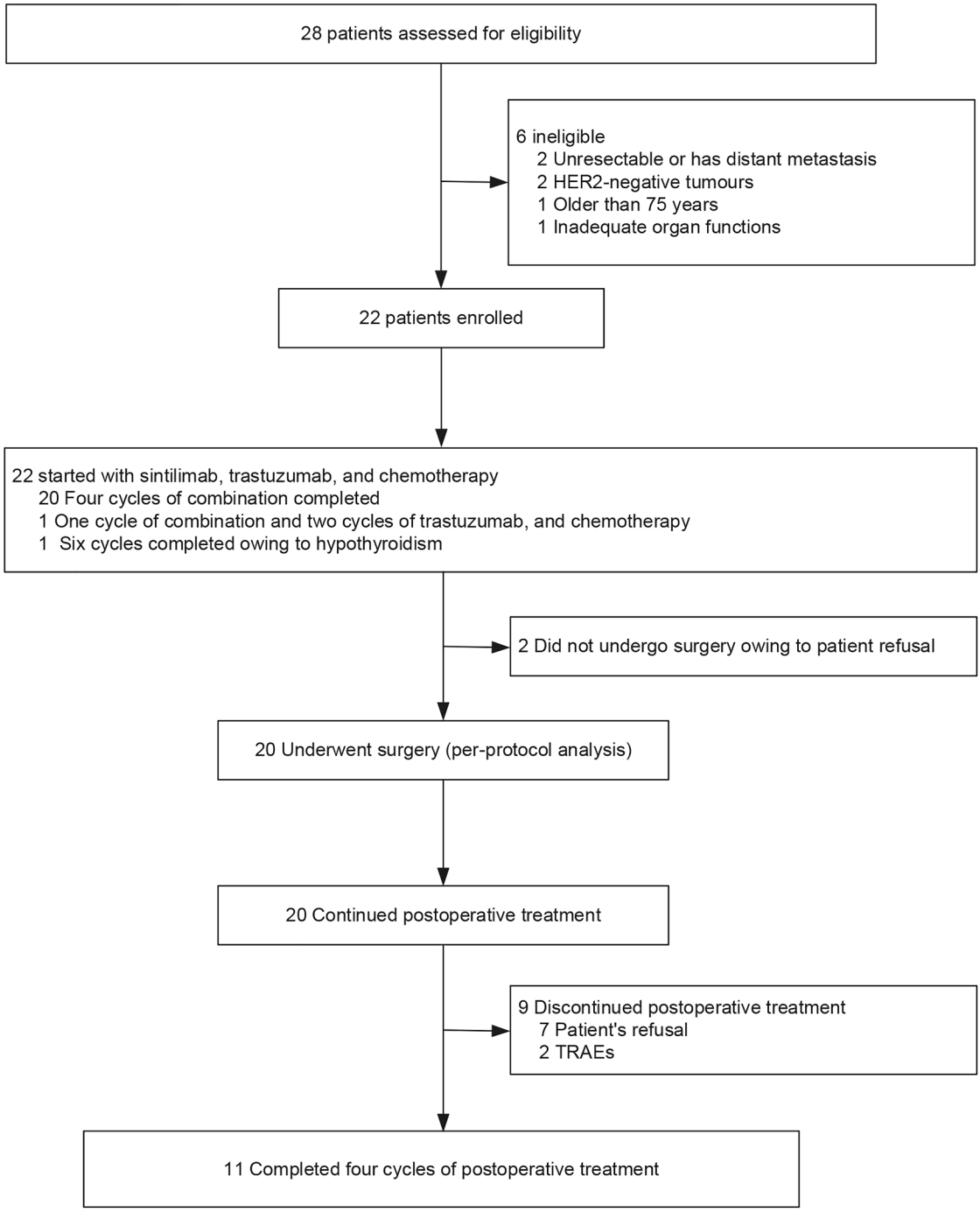

Figure 1. Trial profile

localized HER2-positive gastric and gastro-esophageal junction adenocarcinoma.

RESULTS

Of the 10 patients enrolled in the first stage of the trial, four had a major pathological response (MPR); therefore, we enrolled 12 more patients in the second stage. Between November 3, 2022, and October 18, 2023, 28 patients were screened, of whom six were excluded based on the eligibility criteria (Figure 1). The median age was 61 (interquartile range [IQR], 52–69) years; 16 (73%) were males, and 18 (82%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 0. Of the 22 eligible patients, 45% (10 of 22) had gastro-esophageal

Table 1. Baseline demographic and clinical characteristics (*N* = 22)

Characteristic	Patients, no. (*n* = 22)
Age, years	61 (52–69)
Male	16 (73%)
ECOG performance status	
0	18 (82%)
1	4 (18%)
Primary site	
Gastro-esophageal junction	10 (45%)
Gastric	12 (55%)
HER2 by IHC, FISH, at SYSUCC	
IHC HER2 2+ FISH-positive	7 (32%)
IHC HER2 3+	15 (68%)
Clinical T stage	
cT3	6 (27%)
cT4a	16 (73%)
Clinical N stage	
cN0-1	9 (41%)
cN2-3	13 (59%)
Lauren's classification	
Diffuse	3 (14%)
Intestinal	13 (59%)
Mixed	5 (23%)
Unknown	1 (5%)
PD-L1 CPS	
<1 (negative)	2 (9%)
≥1 (positive)	19 (86%)
<5	12 (55%)
≥5	9 (41%)
Not available	1 (5%)
MMR status	
pMMR	22 (100%)
EBV status	
Positive	1 (5%)
Negative	21 (95%)
Laparoscopic exploration	
Yes	20 (91%)
No	2 (9%)

Data are *n* (%) or median (IQR), unless otherwise indicated. ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; SYSUCC, Sun Yat-sen University Cancer Center; PD-L1, programmed cell death-ligand 1; CPS, combined positive score; MMR, mismatch repair; pMMR, mismatch repair-proficient; EBV, Epstein-Barr virus.

junction adenocarcinoma and 59% (13 of 22) had an intestinal-type histology. All patients had mismatch repair (MMR)-proficient tumors. Nineteen (86%) of the 22 patients had a PD-L1 CPS of 1 or more, and nine (41%) had a PD-L1 CPS of 5 or more. One patient (4.5%) had an Epstein-Barr virus-positive tumor. Diagnostic laparoscopic exploration was performed in 20 (91%) of 22 patients (Table 1).

All 22 eligible patients received at least one cycle of the assigned neoadjuvant sintilimab, trastuzumab, and chemotherapy, of whom 20 (91%) completed the four planned cycles of neoadjuvant therapy. One patient (4.5%) developed immune-related hepatitis after the first cycle of sintilimab, trastuzumab, and chemotherapy and subsequently received two cycles of trastuzumab and chemotherapy. One patient (4.5%) received six cycles of neoadjuvant sintilimab, trastuzumab, and chemotherapy owing to hypothyroidism and patient request (Figure 1). No grade 4 or 5 treatment-related adverse events were recorded during neoadjuvant therapy, while grade 3 treatment-related adverse events were recorded in three (14%) patients (Table S1). The Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 evaluation was performed in 22 patients after the completion of neoadjuvant therapy: complete response was seen in 2 patients (9.1%), partial response in 6 patients (27%), stable disease in 2 patients (9.1%), and it was not evaluable due to the lack of measurable lesions in 12 patients (55%).

Following neoadjuvant treatment, 20 patients (91%) underwent surgery and were subsequently subjected to per-protocol analyses. Two patients declined surgery after neoadjuvant therapy. The median interval between the completion of last cycle of neoadjuvant therapy and surgery was 3 (IQR: 3–4) weeks. Total gastrectomy was performed in 12 (60%) of 20 patients. D2 lymphadenectomy was performed in all 20 patients, and complete R0 resection was performed in 19 patients (95%). Three (15%) patients experienced at least one serious adverse event related to perioperative morbidity, and none died within 30 days of surgery (Table S2).

The primary endpoint is MPR in the intention-to-treat population. In the intention-to-treat population, 12 (55%, 95% confidence interval [CI]: 32–76) patients achieved MPR in primary tumor, and 11 (50%, 95% CI: 28–72) patients achieved pathological complete response in primary tumor and lymph nodes. Pathological outcomes and tumor regression results in the per-protocol population are shown in Table 2. Of the 20 patients who underwent surgery, 12 (60%, 95% CI: 36–81) patients achieved MPR. Eleven (55%, 95% CI: 32–77) of 20 patients achieved pathological complete response in primary tumor and lymph nodes (Table 2; Figure 2A).

All the 20 patients who underwent surgery received adjuvant therapy (Figure 2B). Of these, 10 (50%) patients who achieved pathological complete response received sintilimab and chemotherapy. Two patients whose postoperative HER2 status was negative were administered sintilimab and chemotherapy. One patient who achieved a pathological complete response but developed immune-related hepatitis during the neoadjuvant stage was administered chemotherapy alone. The remaining seven (35%) patients whose post-surgery HER2 status was positive were administered sintilimab, trastuzumab, and chemotherapy. Of the 20 patients, 11 (55%) completed the four planned adjuvant therapy cycles, and seven (35%) and two (10%) stopped receiving treatment because of patient refusal and treatment-related adverse events, respectively (Figure 1 and Tables S3 and S4).

At the data cutoff date on March 11, 2025, the median follow-up time was 22 (IQR: 19–24) months. The treatment times for all

Table 2. Pathological and tumor regression in the per-protocol population (*N* = 20)

Characteristic	Patients, no. (%)
Pathological T stage (ypT)	
ypT0	11 (55%)
ypT1	2 (10%)
ypT2	2 (10%)
ypT3	4 (20%)
ypT4	1 (5%)
Pathological N stage (ypT)	
ypN0	15 (75%)
ypN1	2 (10%)
ypN2	3 (15%)
TRG	
TRG 0	11 (55%)
TRG 1	1 (5%)
TRG 2	5 (25%)
TRG 3	3 (15%)
Combined TRG 0-1	12 (60%)
Tumor residual percentage	
0%	11 (55%)
1%–10%	1 (5%)
11%–100%	8 (40%)
MPR (0%–10%)	12 (60%)

Data are *n* (%). Percentages may not add up to 100 because of rounding. TRG, tumor regression grade; MPR, major pathological response.

the treated patients are shown in Figure 2B. Of the 22 treated patients, 21 (95%) survived (Figure 2B). One patient refused surgery and died of progressive disease; the other patient who refused surgery survived. Two patients suffered recurrence, including one case with loss of HER2 positivity. Overall, the median event-free survival was not reached, and the Kaplan-Meier estimate of the 24-month event-free survival was 73% (95% CI: 46–100; Figure S1A). The median overall survival was not reached, with a 24-month overall survival rate of 96% (95% CI: 87–100; Figure S1B).

Treatment-related adverse events occurred in all patients during perioperative therapy, with nausea, leukopenia, neutropenia, and thrombocytopenia being the most frequent (Table 3). Grade 3 treatment-related adverse events were reported in six (27%) of the 22 patients, and no grade 4 or 5 treatment-related adverse events were observed. Immune-related adverse events were reported in 4 (18%) patients, and all were grade 1 or 2. No deaths were considered treatment related.

In the *post hoc* analysis, we found that tumor regression was comparable irrespective of tumor location, Lauren classification, and PD-L1 CPS. Notably, a MPR was achieved in five (39%) of 13 patients with HER2 immunohistochemistry (IHC) 3+ and in all seven patients with HER2 IHC 2+ fluorescence *in situ* hybridization (FISH)-positive (Figure 2A and Table S5). Of the seven patients with HER2 IHC 2+ FISH-positive, five had gastro-esophageal junction adenocarcinoma, and two had gastric adenocarcinoma.

Subsequently, we conducted RNA sequencing on baseline tumor biopsies and post-treatment samples from 21 patients to assess whether immune cell types within the tumor immune microenvironment (TIME) control the treatment response (Figures 3A and 3B). Of note, the baseline immune components could not predict the treatment response of neoadjuvant treatment (Figure S2). After neoadjuvant treatment, increased levels of CD8 T cells and follicular helper T cells (Tfh) were found in patients achieving MPR than in patients not achieving MPR (Figures 3C and 3D). In addition, increased levels of regulatory T cells (Tregs) and γδT cells were found in patients not achieving MPR than in patients achieving MPR (Figures 3E and 3F). We also conducted immunofluorescence staining and confirmed that Tregs were reduced in patients who achieved MPR yet elevated in those who did not achieve MPR (Figure 3G).

DISCUSSION

In this study, we aimed to evaluate the efficacy and safety of the perioperative anti-PD-1 antibody sintilimab, trastuzumab, and chemotherapy in localized HER2-positive gastric and gastro-esophageal junction adenocarcinoma. Our trial successfully reached its primary endpoint, with 12 (55%) of 22 patients in the intention-to-treat population achieving an MPR. This is a notably high proportion for this tumor type. Furthermore, a complete pathological response was recorded in 11 (50%) of 22 patients. The addition of sintilimab and trastuzumab to chemotherapy did not result in increased surgical or non-surgical adverse events. These findings indicate that the administration of sintilimab, trastuzumab, and chemotherapy in the context of perioperative treatment is safe and effective for the treatment of HER2-positive gastric or gastro-esophageal junction adenocarcinoma.

There is no globally accepted standard of care for patients with operable HER2-positive gastric or gastro-esophageal junction adenocarcinoma.[3,4,18] Based on the results of the FLOT4 trial,[5] the perioperative administration of fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) is recommended for patients with locally advanced HER2-positive disease in non-Asian countries.[3,4] Given the successful results of the RESOLVE trial,[6] the adoption of perioperative S-1 and oxaliplatin is recommended for HER2-positive cases in China.[18] Therefore, in this trial, we used S-1 and oxaliplatin as the backbone of the perioperative chemotherapy regimen.

Tumors with HER2 amplification or overexpression exhibit several unique characteristics. HER2-positive is a predictor of unfavorable survival outcomes and plays a critical role in contributing chemoresistance.[19–21] In terms of the underlying mechanism, perioperative chemotherapy may not be sufficient for locally advanced HER2-positive cases. Several phase 2 trials have sought to incorporate anti-HER2 therapy into perioperative management, resulting in favorable pathological response.[16,17,22] The analysis of preclinical models and tumor samples has shown that trastuzumab can upregulate PD-1 and PD-L1 expression, enhance immune infiltration, and augment HER2-specific T cell responses.[23,24] Oxaliplatin has unique effects on the stimulation and trafficking of dendritic cells, which in turn promote adaptive

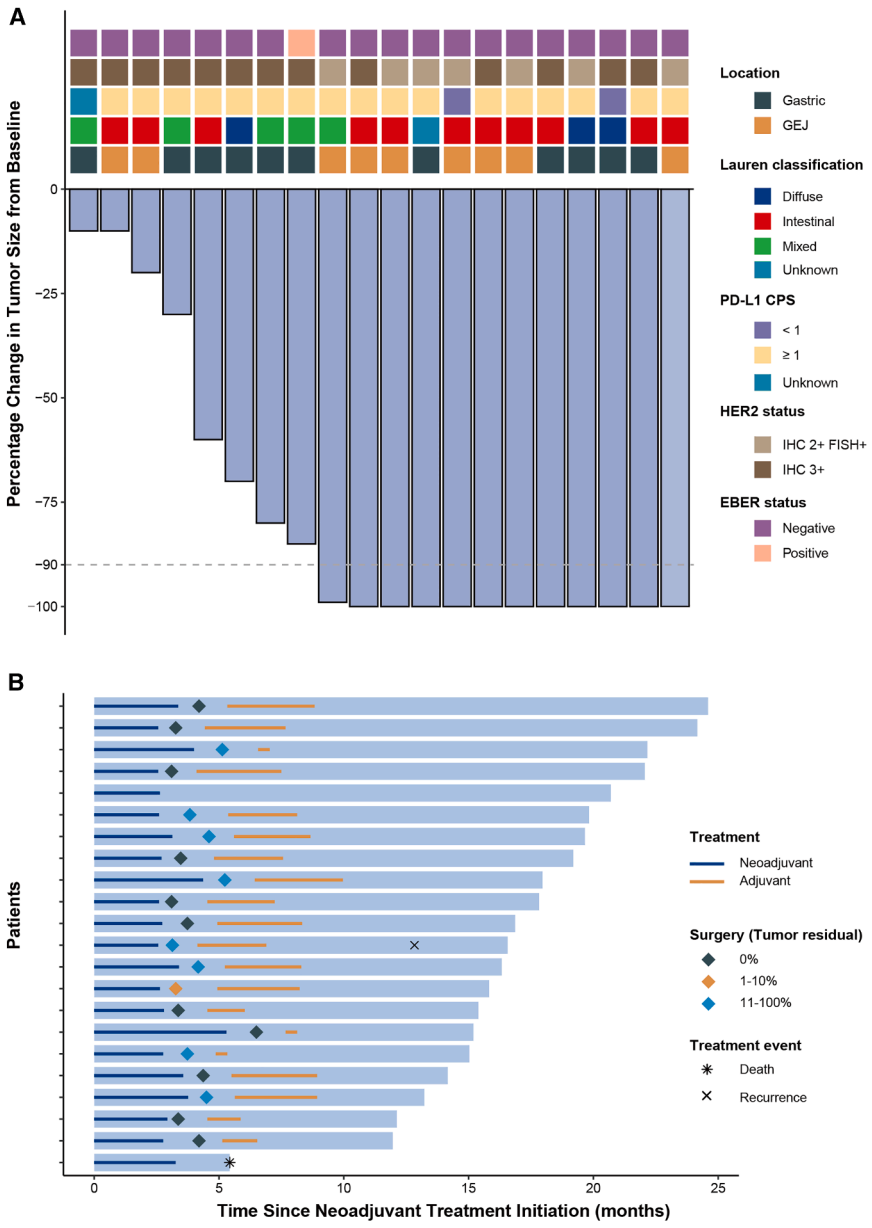

Figure 2. Tumor response and follow-up

(A) Waterfall plot of tumor regression to sintilimab, trastuzumab, and chemotherapy. Patients who refused to surgery are not shown (n = 2).

(B) Swimmer plot showing the treatment events and follow-up in the intent-to-treat population. GEJ, gastro-esophageal junction; PD-L1, programmed cell death-ligand 1; CPS, combined positive score; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; EBV, Epstein-Barr virus.

immunity against tumors.[25,26] The treatment approach of combining dual PD-1 and HER2 blockade with chemotherapy is strongly supported in patients with metastatic HER2-positive gastric and gastro-esophageal junction adenocarcinoma.[10,11] Our trial revealed that the addition of sintilimab and trastuzumab to chemotherapy led to a robust pathological response, with major and complete pathological response rates of 55% and 50%, respectively. These results exceed those achieved with neoadjuvant chemotherapy combined with either anti-PD-1[13–15,27] or anti-HER2 therapy.[16,17] At the 2024 American Society of Clinical Oncology Gastrointestinal Cancers Symposium, a randomized phase 2 trial reported the preliminary efficacy of adding atezolizumab to treatment with trastuzumab and chemotherapy compared to trastuzumab and chemotherapy in the perioperative setting.[28] The trial reported a complete pathological response rate of 38% versus 14%, which is worth anticipating.

To investigate the characteristics that predicted primary resistance to this investigational therapy, we performed several subgroup analyses. The PD-L1 CPS was predictive of survival benefits in trials of first-line anti-PD-1 antibodies plus chemotherapy for advanced gastric carcinomas.[27,29] However, no improvement in the pathological response was observed in patients with high PD-L1 CPS in this study population, which is consistent with the findings of several trials in the perioperative setting.[13,15] A previous study highlighted that HER2 amplification using next-generation sequencing is predictive of the therapeutic benefits of trastuzumab-based therapy.[30] Intriguingly, our trial found that patients with HER2 IHC 2+ FISH positivity demonstrated a higher pathological response than those with HER2 IHC 3+ positivity. Notably, our study found distinct anatomical distribution patterns, with higher proportion of HER2 amplification in tumor with gastro-esophageal junction adenocarcinoma, highlighting the attention of HER2 testing for this cancer types. In addition, we observed two cases in which HER2 positivity was lost following four cycles of neoadjuvant therapy, and an MPR was not achieved. This suggests that the loss of HER2 positivity can contribute to acquired resistance to trastuzumab-based therapy.[31,32]

In this study, the TIME dynamic change after perioperative dual PD-1 and HER2 blockade was assessed. Consistent with prior research,[33] patients who achieved MPR exhibited elevated levels of CD8 T cells and Tfh cells. From a mechanistic standpoint, PD-1 suppresses Treg activity, and PD-1 blockade leads to enhanced Treg activation.[34] In our study, we found that, after neoadjuvant treatment, non-responders exhibited an increase in Tregs, while responders showed a decrease in Tregs. γδT cells constitute a minor subset of immune cells that have been

Table 3. Summary of adverse events (*N* = 22)

	No. (%)			
TRAE	Any grade	Grade 1	Grade 2	Grade 3
Any events	22 (100%)	6 (27%)	10 (45%)	6 (27%)
TRAEs leading to treatment discontinuation	2 (9%)	0	1 (5%)	1 (5%)
Nausea	14 (64%)	12 (55%)	2 (9%)	0
Vomit	10 (45%)	9 (41%)	1 (5%)	0
Diarrhea	11 (50%)	7 (32%)	4 (18%)	0
Decreased appetite	9 (41%)	9 (41%)	0	0
Infusion reaction	1 (5%)	0	2 (9%)	0
Pneumonia	1 (5%)	0	2 (9%)	0
Edema	1 (5%)	1 (5%)	0	0
Peripheral neuropathy	4 (18%)	2 (9%)	1 (5%)	1 (5%)
Increased ALT or AST	10 (45%)	7 (32%)	2 (9%)	1 (5%)
Leukopenia	14 (64%)	4 (18%)	9 (41%)	1 (5%)
Neutropenia	14 (64%)	3 (14%)	8 (36%)	3 (14%)
Thrombocytopenia	14 (64%)	3 (14%)	8 (36%)	3 (14%)
Anemia	4 (18%)	3 (14%)	1 (5%)	0
Immune-related AE	4 (18%)	2 (9%)	2 (9%)	0
Rash	1 (5%)	1 (5%)	0	0
Enteritis	1 (5%)	0	1 (5%)	0
Hepatitis	1 (5%)	0	1 (5%)	0
Hypothyroidism	1 (5%)	1 (5%)	0	0

Data are *n* (%). TRAE, treatment-related adverse event; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

relatively under-researched, yet their potential has been acknowledged in the context of cancer immunotherapy.[35] We also found that, after neoadjuvant treatment, non-responders demonstrated an increase in γδT cells, indicating that γδT cells were associated with possibility of drug resistance. However, the mechanism by which γδT cells regulate drug resistance remains under investigation.

Although all patients experienced treatment-related adverse events, no grade 4 or 5 adverse events were associated with the treatment. The occurrence of grade 3 events was comparable to that observed with a combination of trastuzumab or anti-PD-1 therapy and chemotherapy.[13,15,16] No new safety concerns were observed in this trial, and the perioperative morbidity was acceptable, with no 30-day mortality.

Limitations of the study

The limitations of this trial include its single-arm design and modest sample size. Furthermore, the fact that this trial was conducted at a single center may limit the generalizability of the findings. We conducted a multicenter randomized trial (NEOSUMMIT-02B) to further validate the efficacy of the perioperative anti-PD-1 antibodies sintilimab and trastuzumab and chemotherapy in localized HER2-positive gastric and gastro-esophageal junction adenocarcinoma (Chinese Clinical Trial Registry, identifier: ChiCTR2400081665). In addition, the MPR was assessed through a local review. Moreover, the biomarker analysis is preliminary; additional analyses and experiments would ideally be necessary to elucidate potential mechanisms. Finally, a continued follow-up is required for the survival data to mature and evaluate whether the improvement in the pathological response can translate into a survival benefit.

In conclusion, perioperative sintilimab, trastuzumab, and chemotherapy have promising antitumor activity with a manageable safety profile and might be promising options for patients with localized HER2-positive gastric and gastro-esophageal junction adenocarcinoma.

RESOURCE AVAILABILITY

Lead contact

Requests for further information, resources, and reagents should be directed to and will be fulfilled by the lead contact, Yuan-Fang Li (liyuanf@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The raw sequencing data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA008961), which are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.
- No custom computer codes are reported in this paper. The code for data analysis is available upon request.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

This study is sponsored by Innovent Biologics. The authors thank the patients who participated in this study and their families. This research was supported by the Noncommunicable Chronic Diseases-National Science and Technology Major Project (2023ZD0501500 to S.-Q.Y.), Young Talents Program of Sun Yat-sen University Cancer Center (YTP-SYSUCC-0098 to R.-C.N.), Guangdong Basic and Applied Basic Research Foundation (2025A1515010501 to Y.-F.L. and 2025A1515011488 to R.-C.N.), and Beijing Xisike Clinical Oncology Research Foundation (Y-2022METAZMS-0123 to Y.-F.L., Y-HR2022MS-0324 to S.-Q.Y., and Y-2023AZMETQN-0017 to R.-C.N.). The funders played no role in the study's design, conduct, or reporting.

AUTHOR CONTRIBUTIONS

Y.-F.L., S.-Q.Y., Y.-M.C., and R.-C.N. were responsible for study design and planning. M.-Y.C., R.-C.N., X.-J.C., C.-C.L., B.-W.Z., W.W., H.-B.Q., G.-M.C., Z.-M.L., and J.C. were responsible for data acquisition. M.-Y.C., R.-C.N., X.-J.C., J.-L.D., D.-S.Z., Y.-B.C., Z.-W.Z., and Y.-M.C. analyzed the data and interpreted the results. M.-Y.C., R.-C.N., X.-J.C., Y.-F.L., S.-Q.Y., and Y.-M.C. drafted the manuscript. All authors contributed to critically reviewing or revising the manuscript and gave final approval for submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Human subjects and ethical approval

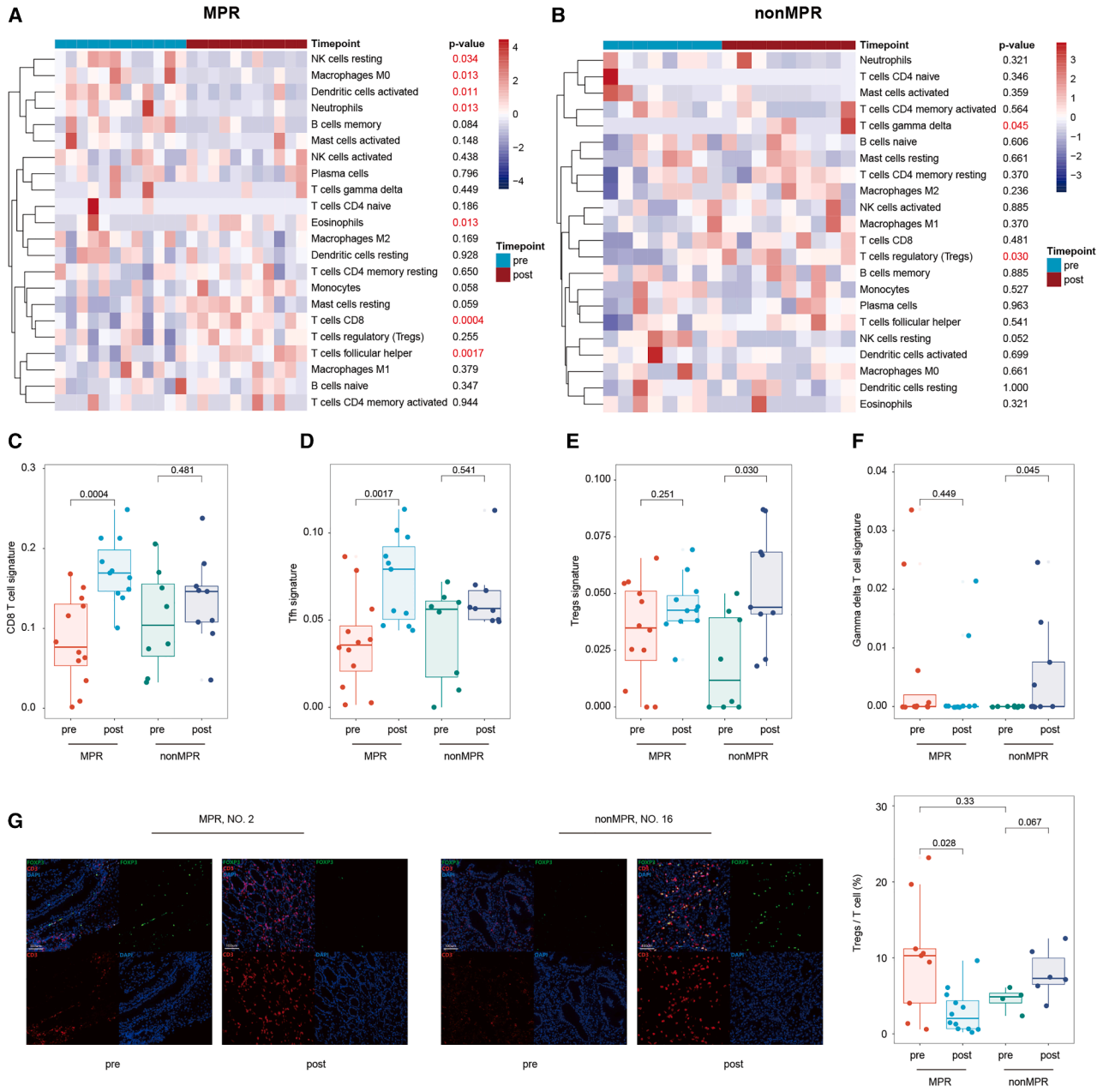

Figure 3. Immune cell landscape of patients at baseline and after neoadjuvant treatment

(A and B) Heatmaps depicting 22 immune cell types at baseline and after neoadjuvant treatment in patients who achieved an MPR (A) and those who did not (B).

(C–F) Analysis of CD8 T cell (C), follicular helper T cell (Tfh) (D), regulatory T cell (Tregs) (E), and γδT cell (F) infiltration at baseline and after neoadjuvant treatment in patients who achieved an MPR (left, *N* = 12) and those who did not (right, *N* = 9).

(G) Immunofluorescence staining and quantification of Tregs at baseline and after neoadjuvant treatment in patients who achieved an MPR (*N* = 12) and those who did not (*N* = 6).

MPR, major pathological response. Wilcoxon rank-sum test.

- METHOD DETAILS
  - Patients and study design
  - Treatment
  - Assessments and outcomes
  - Sample size calculation
  - Biomarker analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2025.102190.

Received: January 14, 2025
Revised: April 17, 2025
Accepted: May 21, 2025
Published: June 17, 2025

REFERENCES

1. Bray, F., Laversanne, M., and Sung, H. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. *74*, 229–263.
2. Sano, T., Coit, D.G., Kim, H.H., Roviello, F., Kassab, P., Wittekind, C., Yamamoto, Y., and Ohashi, Y. (2017). Proposal of a new stage grouping of gastric cancer for TNM classification: International Gastric Cancer Association staging project. Gastric Cancer *20*, 217–225.
3. Ajani, J.A., D'Amico, T.A., Bentrem, D.J., Chao, J., Cooke, D., Corvera, C., Das, P., Enzinger, P.C., Enzler, T., Fanta, P., et al. (2022). Gastric Cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. J. Natl. Compr. Canc. Netw. *20*, 167–192.
4. Lordick, F., Carneiro, F., Cascinu, S., Fleitas, T., Haustermans, K., Piessen, G., Vogel, A., and Smyth, E.C.; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org (2022). Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann. Oncol. *33*, 1005–1020.
5. Al-Batran, S.E., Homann, N., Pauligk, C., Goetze, T.O., Meiler, J., Kasper, S., Kopp, H.G., Mayer, F., Haag, G.M., Luley, K., et al. (2019). Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. Lancet *393*, 1948–1957.
6. Zhang, X., Liang, H., Li, Z., Xue, Y., Wang, Y., Zhou, Z., Yu, J., Bu, Z., Chen, L., Du, Y., et al. (2021). Perioperative or postoperative adjuvant oxaliplatin with S-1 versus adjuvant oxaliplatin with capecitabine in patients with locally advanced gastric or gastro-oesophageal junction adenocarcinoma undergoing D2 gastrectomy (RESOLVE): an open-label, superiority and non-inferiority, phase 3 randomised controlled trial. Lancet Oncol. *22*, 1081–1092.
7. Cancer Genome Atlas Research Network (2014). Comprehensive molecular characterization of gastric adenocarcinoma. Nature *513*, 202–209.
8. Van Cutsem, E., Bang, Y.J., Feng-Yi, F., Xu, J.M., Lee, K.W., Jiao, S.C., Chong, J.L., López-Sanchez, R.I., Price, T., Gladkov, O., et al. (2015). HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer *18*, 476–484.
9. Bang, Y.J., Van Cutsem, E., Feyereislova, A., Chung, H.C., Shen, L., Sawaki, A., Lordick, F., Ohtsu, A., Omuro, Y., Satoh, T., et al. (2010). Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet *376*, 687–697.
10. Janjigian, Y.Y., Kawazoe, A., Yañez, P., Li, N., Lonardi, S., Kolesnik, O., Barajas, O., Bai, Y., Shen, L., Tang, Y., et al. (2021). The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature *600*, 727–730.
11. Janjigian, Y.Y., Kawazoe, A., Bai, Y., Xu, J., Lonardi, S., Metges, J.P., Yanez, P., Wyrwicz, L.S., Shen, L., Ostapenko, Y., et al. (2023). Pembrolizumab plus trastuzumab and chemotherapy for HER2-positive gastric or gastro-oesophageal junction adenocarcinoma: interim analyses from the phase 3 KEYNOTE-811 randomised placebo-controlled trial. Lancet *402*, 2197–2208.
12. Janjigian, Y.Y., Kawazoe, A., Bai, Y., Xu, J., Lonardi, S., Metges, J.P., Yañez, P., Wyrwicz, L.S., Shen, L., Ostapenko, Y., et al. (2024). Pembrolizumab in HER2-Positive Gastric Cancer. N. Engl. J. Med. *391*, 1360–1362.
13. Shitara, K., Rha, S.Y., Wyrwicz, L.S., Oshima, T., Karaseva, N., Osipov, M., Yasui, H., Yabusaki, H., Afanasyev, S., Park, Y.K., et al. (2024). Neoadjuvant and adjuvant pembrolizumab plus chemotherapy in locally advanced gastric or gastro-oesophageal cancer (KEYNOTE-585): an interim analysis of the multicentre, double-blind, randomised phase 3 study. Lancet Oncol. *25*, 212–224.
14. Janjigian, Y.Y., Al-Batran, S.E., Wainberg, Z.A., Van Cutsem, E., Molena, D., Muro, K., Hyung, W., Wyrwicz, L., Oh, D.Y., Omori, T., et al. (2023). Pathological complete response (pCR) to durvalumab plus 5-fluorouracil, leucovorin, oxaliplatin and docetaxel (FLOT) in resectable gastric and gastroesophageal junction cancer (GC/GEJC): Interim results of the global, phase III MATTERHORN study. Ann. Oncol. *34*, S1315–S1316.
15. Yuan, S.Q., Nie, R.C., Jin, Y., Liang, C.C., Li, Y.F., Jian, R., Sun, X.W., Chen, Y.B., Guan, W.L., Wang, Z.X., et al. (2024). Perioperative toripalimab and chemotherapy in locally advanced gastric or gastro-esophageal junction cancer: a randomized phase 2 trial. Nat. Med. *30*, 552–559.
16. Rivera, F., Izquierdo-Manuel, M., García-Alfonso, P., Martínez de Castro, E., Gallego, J., Limón, M.L., Alsina, M., López, L., Galán, M., Falcó, E., et al. (2021). Perioperative trastuzumab, capecitabine and oxaliplatin in patients with HER2-positive resectable gastric or gastro-oesophageal junction adenocarcinoma: NEOHX phase II trial. Eur. J. Cancer *145*, 158–167.
17. Hofheinz, R., Hegewisch-Becker, S., Thuss-Patience, P.C., Kunzmann, V., Fuchs, M., Graeven, U., Homann, N., Heinemann, V., Pohl, M., Tannapfel, A., and Al-Batran, S.E. (2014). HER-FLOT: Trastuzumab in combination with FLOT as perioperative treatment for patients with HER2-positive locally advanced esophagogastric adenocarcinoma: A phase II trial of the AIO Gastric Cancer Study Group. J. Clin. Oncol. *32*, 4073.
18. Wang, F.H., Zhang, X.T., Tang, L., Wu, Q., Cai, M.Y., Li, Y.F., Qu, X.J., Qiu, H., Zhang, Y.J., Ying, J.E., et al. (2024). The Chinese Society of Clinical Oncology (CSCO): Clinical guidelines for the diagnosis and treatment of gastric cancer. Cancer Commun. *44*, 127–172.
19. Gao, X., Zhao, L., Zhang, N., Han, W., Liu, K., Yan, J., Chen, L., Pan, Y., Li, R., Li, W., et al. (2023). Impact of HER2 on prognosis and benefit from adjuvant chemotherapy in stage II/III gastric cancer patients: a multicenter observational study. Int. J. Surg. *109*, 1330–1341.
20. Knuefermann, C., Lu, Y., Liu, B., Jin, W., Liang, K., Wu, L., Schmidt, M., Mills, G.B., Mendelsohn, J., and Fan, Z. (2003). HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene *22*, 3205–3212.
21. Chevallier, P., Robillard, N., Wuilleme-Toumi, S., Méchinaud, F., Harousseau, J.L., and Avet-Loiseau, H. (2004). Overexpression of Her2/neu is observed in one third of adult acute lymphoblastic leukemia patients and is associated with chemoresistance in these patients. Haematologica (Roma) *89*, 1399–1401.
22. Hofheinz, R.D., Merx, K., Haag, G.M., Springfeld, C., Ettrich, T., Borchert, K., Kretzschmar, A., Teschendorf, C., Siegler, G., Ebert, M.P., et al. (2022). FLOT Versus FLOT/Trastuzumab/Pertuzumab Perioperative Therapy of Human Epidermal Growth Factor Receptor 2-Positive Resectable Esophagogastric Adenocarcinoma: A Randomized Phase II Trial of the AIO EGA Study Group. J. Clin. Oncol. *40*, 3750–3761.
23. Taylor, C., Hershman, D., Shah, N., Suciu-Foca, N., Petrylak, D.P., Taub, R., Vahdat, L., Cheng, B., Pegram, M., Knutson, K.L., and Clynes, R. (2007). Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. Clin. Cancer Res. *13*, 5133–5143.
24. Park, S., Jiang, Z., Mortenson, E.D., Deng, L., Radkevich-Brown, O., Yang, X., Sattar, H., Wang, Y., Brown, N.K., Greene, M., et al. (2010). The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell *18*, 160–170.
25. Ghiringhelli, F., Apetoh, L., Tesniere, A., Aymeric, L., Ma, Y., Ortiz, C., Vermaelen, K., Panaretakis, T., Mignot, G., Ullrich, E., et al. (2009). Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat. Med. *15*, 1170–1178.
26. Tesniere, A., Schlemmer, F., Boige, V., Kepp, O., Martins, I., Ghiringhelli, F., Aymeric, L., Michaud, M., Apetoh, L., Barault, L., et al. (2010).

Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene *29*, 482–491.

27. Lorenzen, S., Götze, T.O., Thuss-Patience, P., Biebl, M., Homann, N., Schenk, M., Lindig, U., Heuer, V., Kretzschmar, A., Goekkurt, E., et al. (2024). Perioperative Atezolizumab Plus Fluorouracil, Leucovorin, Oxaliplatin, and Docetaxel for Resectable Esophagogastric Cancer: Interim Results From the Randomized, Multicenter, Phase II/III DANTE/IKF-s633 Trial. J. Clin. Oncol. *42*, 410–420.
28. Peng, Z., Zhang, X., Liang, H., Zheng, Z., Wang, Z., Liu, H., Hu, J., Sun, Y., Zhang, Y., Yan, H., et al. (2024). Atezolizumab and trastuzumab plus chemotherapy in patients with HER2+ locally advanced resectable gastric cancer or adenocarcinoma of the gastroesophageal junction: A multicenter, randomized, open-label phase II study. J. Clin. Oncol. *42*, 312.
29. Janjigian, Y.Y., Shitara, K., Moehler, M., Garrido, M., Salman, P., Shen, L., Wyrwicz, L., Yamaguchi, K., Skoczylas, T., Campos Bragagnoli, A., et al. (2021). 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 *398*, 27–40.
30. Janjigian, Y.Y., Sanchez-Vega, F., Jonsson, P., Chatila, W.K., Hechtman, J.F., Ku, G.Y., Riches, J.C., Tuvy, Y., Kundra, R., Bouvier, N., et al. (2018). Genetic Predictors of Response to Systemic Therapy in Esophagogastric Cancer. Cancer Discov. *8*, 49–58.
31. Pietrantonio, F., Caporale, M., Morano, F., Scartozzi, M., Gloghini, A., De Vita, F., Giommoni, E., Fornaro, L., Aprile, G., Melisi, D., et al. (2016). HER2 loss in HER2-positive gastric or gastroesophageal cancer after trastuzumab therapy: Implication for further clinical research. Int. J. Cancer *139*, 2859–2864.
32. Seo, S., Ryu, M.H., Park, Y.S., Ahn, J.Y., Park, Y., Park, S.R., Ryoo, B.Y., Lee, G.H., Jung, H.Y., and Kang, Y.K. (2019). Loss of HER2 positivity after anti-HER2 chemotherapy in HER2-positive gastric cancer patients: results of the GASTric cancer HER2 reassessment study 3 (GASTHER3). Gastric Cancer *22*, 527–535.
33. Kim, R., An, M., Lee, H., Mehta, A., Heo, Y.J., Kim, K.M., Lee, S.Y., Moon, J., Kim, S.T., Min, B.H., et al. (2022). Early Tumor-Immune Microenvironmental Remodeling and Response to First-Line Fluoropyrimidine and Platinum Chemotherapy in Advanced Gastric Cancer. Cancer Discov. *12*, 984–1001.
34. Tay, C., Tanaka, A., and Sakaguchi, S. (2023). Tumor-infiltrating regulatory T cells as targets of cancer immunotherapy. Cancer Cell *41*, 450–465.
35. Wiesheu, R., and Coffelt, S.B. (2024). From backstage to the spotlight: gammadeltaT cells in cancer. Cancer Cell *42*, 1637–1642.
36. Simon, R. (1989). Optimal two-stage designs for phase II clinical trials. Control. Clin. Trials *10*, 1–10.
37. Newman, A.M., Steen, C.B., Liu, C.L., Gentles, A.J., Chaudhuri, A.A., Scherer, F., Khodadoust, M.S., Esfahani, M.S., Luca, B.A., Steiner, D., et al. (2019). Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat. Biotechnol. *37*, 773–782.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibody		
CD3	ZSGB-Bio	Cat #ZA-0503; RRID:AB_3665339
FOXP3	Abcam	Cat #ab215206; RRID:AB_2860568
Biological samples		
Pre-treatment biopsy samples	This study	N/A
Post-treatment resected samples	This study	N/A
Deposited data		
RNAseq data	This study	GSA: HRA008961
Software and algorithms		
R (version 4.3.2)	R Development Core Team, 2008	www.r-project.org
PASS software (version 2021)	NCSS, LLC	https://www.ncss.com/software/pass/

EXPERIMENTAL MODELS AND STUDY PARTICIPANT DETAILS

Human subjects and ethical approval

This study included human participants from China. The study protocol and amendments were approved by the Institutional Review Board of the SYSUCC. The study was performed in accordance with the protocol, Good Clinical Practice guidelines, and Declaration of Helsinki. All patients provided written informed consent to participate in the study. This trial was registered in the Chinese Clinical Trial Registry (chictr.org.cn; identifier: ChiCTR2200058732). HER2-positive patients with localised advanced gastric or gastro-oesophageal junction adenocarcinoma were enrolled. Demographic information, including age and gender, was provided in Table 1.

METHOD DETAILS

Patients and study design

This investigator-initiated, open-label, single-arm, phase 2 trial was conducted at the Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China. Eligible patients had histologically confirmed HER2-positive (defined as immunohistochemistry [IHC] 3+ or IHC 2+ and HER2:CEP17 fluorescence *in situ* hybridisation ratio [FISH] $\geq$ 2.0) and clinical stage cT3-4aNanyM0 or cT1-2N + M0 gastric or gastro-oesophageal junction adenocarcinoma according to the eighth edition of the International Union against Cancer tumour-node-metastasis classification. HER2 status was evaluated using a monoclonal antibody against HER2/neu (clone CB-11, USA), and HER2 fluorescent *in situ* hybridisation was performed in cases with HER2 equivocal (2+ intensity) using the Vysis PathVysion HER-2/neu DNA Probe Kit (Dako, Denmark). The clinical stage was determined by examination, oesophagogastroduodenoscopy, and computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, and pelvis. Before enrollment, diagnostic laparoscopy was recommended to exclude occult peritoneal metastases. The key inclusion criteria were age 18 years or more; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 with no surgical contraindication, life expectancy of 3 months or longer, adequate bone marrow reserve (leukocyte count $\geq$4000/μL, neutrophil count $\geq$1500/μL, platelet count $\geq 10\times10^4$/μL, and hemoglobin $\geq$9.0 g/dL), and normal hepatic (bilirubin $\leq$1.5 times the upper limit of normal, aspartate aminotransferase and alanine aminotransferase $\leq$2.5 times the upper limit of normal), renal (creatinine $\leq$1.5 times the upper limit of normal or serum clearance rate >60 mL/min) and coagulation (international normalised ratio and activated partial thromboplastin time $\leq$1.5 times the upper limit of normal) functions. The major exclusion criteria were previous chemotherapy, radiotherapy, targeted therapy, immunotherapy, or surgical treatment; history of other malignancies within the last 5 years (except for completely resected basal cell carcinoma, stage I squamous cell carcinoma, carcinoma *in situ*, intramucosal carcinoma, and superficial bladder cancer); history of HIV infection, active tuberculosis, or hepatitis B or C; and allergy to the investigational medicine. A full list of inclusion and exclusion criteria can be found in the study protocol (Supplementary protocol).

Treatment

In the neoadjuvant phase, eligible patients were administered 200 mg of sintilimab intravenously once every 3 weeks for four cycles. In addition, all patients received trastuzumab at a dose of 6 mg/kg intravenously once every 3 weeks, following an initial loading dose of 8 mg/kg, oxaliplatin (130 mg/m^2 administered intravenously once every 3 weeks), and S-1 (40–60 mg orally twice daily for 2 weeks

in each 3-week cycle) for four cycles. Surgery was performed within 2–4 weeks of the last cycle of the neoadjuvant phase. In the adjuvant phase, patients were scheduled to receive sintilimab (200 mg administered intravenously once every 3 weeks), oxaliplatin (130 mg/m^2 administered intravenously once every 3 weeks), and S-1 (40–60 mg orally twice daily for 2 weeks in each 3-week cycle) for four cycles. If the HER2 status of the surgical sample was positive, patients would receive trastuzumab (6 mg/kg, administered intravenously once every 3 weeks) for four cycles. Dose reduction was not allowed for sintilimab or trastuzumab treatment; patients were permitted a maximum of two reductions in chemotherapy dosage (Supplementary protocol).

Assessments and outcomes

Imaging assessments by CT or MRI were performed after completion of cycles two and four of neoadjuvant treatment. The patients who underwent surgery were scheduled to undergo repeat scans every 3 months during the first 3 years, every 6 months for 5 years, and annually thereafter. The patients who did not undergo surgery were followed-up every 3 months. Radical response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). The type of gastrectomy was determined by proficient abdominal surgeons based on tumor location, and D2 lymphadenectomy was recommended in this study. An experienced surgeon (YFL) assessed the surgical quality.

The MMR status was evaluated using IHC for MLH1, PMS2, MSH2, and MSH6. PD-L1 expression was assessed using an IHC kit with the 22C3 pharmDx assay (Agilent Technologies, Santa Clara, CA, USA) based on the combined positive score (CPS), which was defined as the number of PD-L1-positive tumor cells (partial or complete membrane staining), lymphocytes, and macrophages (membrane staining, intracellular staining, or both) divided by the total number of viable tumor cells multiplied by 100. Epstein-Barr virus status was determined by EBER using an *in situ* hybridisation (ISH) kit (ISH-7001; Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China; Leica Biosystems, Newcastle, UK). Tumor samples were reviewed and evaluated by local pathologists (JLD and MYC). The adverse events and laboratory abnormalities were routinely recorded during the treatment for up to 30 days. The surgical complications were assessed using the Clavien–Dindo classification within 30 days of surgery.

The primary endpoint was major pathological response, defined as 10% or less of residual viable tumor cells at the time of definitive surgery in the intention-to-treat population. The intention-to-treat population included all patients, regardless of the treatment received or deviations from the protocol. The per-protocol population was defined as patients who received at least one dose of treatment, underwent surgery, and showed no major deviations from the protocol. Secondary endpoints included pathological complete response (defined as no residual viable tumor cells at the time of definitive surgery), pathological complete response (TRG 0) or near-complete response (TRG 1) according to the scoring system for tumor response of NCCN Guidelines for Gastric Cancer,[3] margin-free resection (R0), objective response rate (proportion of patients with partial or complete response per RECIST version 1.1 by investigator), recurrence-free survival (time from the first cycle of treatment to disease recurrence or metastasis, or death from any cause), event-free survival (time from the first cycle of treatment to disease progression, local or distant recurrence, or death from any cause), overall survival (time from the first cycle of treatment to death from any cause), surgical safety, treatment-related adverse events assessed per the CTCAE version 5.0, and quality of life.

Sample size calculation

The planned sample size was 22 patients based on Simon's optimal two-stage design (one-sided α 5% and power 80%; PASS 2021 software).[36] The major pathological response rate to neoadjuvant chemotherapy for locally advanced gastric cancer in the phase 2 NEOSUMMIT-01 trial was 22%,[15] regardless of HER2 status. The patients in our trial were HER2-positive, potentially leading to a decreased pathological response. Therefore, the major pathological response rate could be lower than 22%. In this trial, we considered that an additional increase of at least 25% in the major pathological response was essential to indicate a clinically meaningful antitumour activity. Therefore, the threshold for a major pathological response was set to 20%, and the expected major pathological response was 45%. Ten patients were enrolled in the first stage of the study. If three or more patients showed a major pathological response, 12 more patients were enrolled in the second stage; otherwise, the study would not have advanced to the second stage, and the trial would have been discontinued. If a major pathological response was observed in eight or more of 22 patients, it would indicate that the primary endpoint had been achieved.

Biomarker analysis

We conducted RNA sequencing on baseline tumor biopsies and post-treatment samples from 21 patients to evaluate whether distinct immune cell components within the TIME control the treatment response. The expression matrix of RNA sequencing was normalized to transcripts per kilobase million (TPM) format. Using the CIBERSORTx (https://cibersortx.stanford.edu/) algorithm,[37] we estimated a total of 22 immune cell types. Subjects were deemed eligible for subsequent stages of rigorous analysis only if they exhibited a statistically significant empirical *p*-values below the threshold of 0.05.

We further conducted multi-color immunofluorescence staining on baseline tumor biopsies and post-treatment samples from 18 patients. For multi-color immunofluorescence staining, tissue samples were fixed in formalin, embedded in paraffin, and 4μm sectioned. The tissue sections were deparaffinized in xylene and rehydrated by serial passage through graded concentrations of ethanol. Endogenous peroxidase in tissues was blocked with Endogenous Peroxidase Blocking Buffer for 10 min. Heat-induced epitope retrieval was performed for 10 min at 95°C in Tris–EDTA pH 8.0 buffer or EDTA pH 9.0 buffer. Sections were washed in cold running tap water and Tris-buffered saline–0.05% Tween 20 (TBST) and blocked with blocking Buffer for 20 min. Specimens

were incubated with primary antibodies specific for the following proteins:CD3 (diluted at 1:100, ZA-0503, ZSGB-BIO), FOXP3 (diluted at 1:100, ab20034, Abcam). Then, sections were incubated with polymer HRP Mouse + Rabbit (PANOVUE) for 30 min, followed by incubation with an Opal fluorophore from Opal Multiplex Kit (PANOVUE) for 20 min. After washing in cold running tap water and TBST, the process of staining and antibody removal was repeated using a different Opal fluorophore. Finally, the samples were mounted with Antifade Mounting Medium and DAPI (PANOVUE).

QUANTIFICATION AND STATISTICAL ANALYSIS

Patient characteristics, safety, pathological, and tumor regression data are represented using frequency and percentage for categorical variables and median and interquartile range (IQR) for continuous variables. The intention-to-treat population was assigned to analyze the primary endpoints of major pathological responses, non-surgical and serious adverse events, and survival outcomes. The per-protocol population was assigned to analyze surgical and pathological results, and adverse surgical events. The 95% confidence intervals (CIs) of the major pathological response rates were calculated using the Clopper–Pearson method, and event-free survival and overall survival were estimated using the Kaplan–Meier method and evaluated with 95% CIs calculated using the Greenwood formula. Post hoc analyses were conducted to evaluate tumor regression between groups defined by tumor location (gastric vs. gastro-oesophageal junction), Lauren classification (intestinal vs. non-intestinal), HER2 status (IHC 3+ vs. IHC 2+ FISH-positive), and PD-L1 CPS (<1 vs. $\geq$ 1; <5 vs. $\geq$ 5). Categorical variables between groups were compared using the χ^2 test or Fisher's exact test. Continuous variables between groups were compared using the Wilcoxon rank-sum test. All p-values were two-sided, and statistical significance was set at $p < 0.05$. Statistical analyses were performed using R version 4.3.2.

ADDITIONAL RESOURCES

Chictr.org.cn: ChiCTR2200058732.