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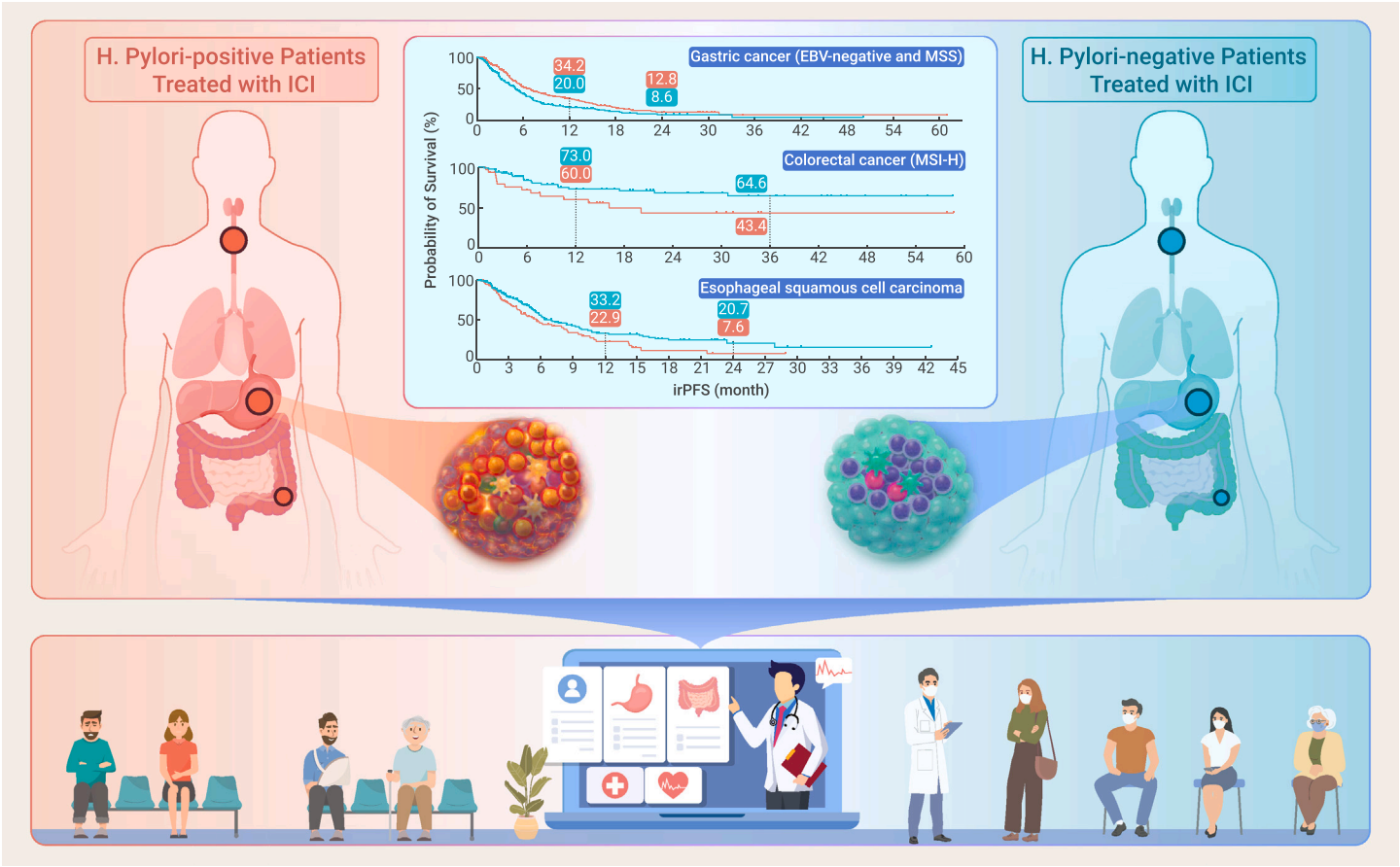
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- *Helicobacter pylori* infection is a favorable factor for gastric cancer immunotherapy by shaping the "hot" tumor microenvironment.
- *H. pylori* infection is unfavorable for immunotherapy in DNA mismatch repair-deficient/microsatellite instability-high colon adenocarcinoma and esophageal squamous cell cancer.
- This study highlights the importance of the test for *H. pylori* infection in cancer patients treated with immunotherapy.

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Helicobacter pylori infection is associated with the risk of gastrointestinal (GI) cancers; however, its impact on immunotherapy for GI cancers remains uncertain. In this study, we included 10,122 patients who underwent ^{13}C-urea breath tests. Among 636 patients with Epstein-Barr virus–negative microsatellite-stable gastric cancer (GC) who were treated with anti-PD-1/PD-L1 therapy, *H. pylori*–positive patients exhibited significantly longer immune-related progression-free survival (irPFS) compared with *H. pylori*–negative patients (6.97 months versus 5.03 months, $p < 0.001$, hazard ratio [HR] 0.76, 95% confidence interval [CI] 0.62–0.95, $p = 0.015$). Moreover, the *H. pylori*–positive group demonstrated a trend of 4 months longer median immune-related overall survival (irOS) than the *H. pylori*–negative group. *H. pylori*–positive GC displayed higher densities of PD-L1$^+$ cells and nonexhausted CD8$^+$ T cells, indicative of a "hot" tumor microenvironment. Transcriptomic analysis revealed that *H. pylori*–positive GC shared molecular characteristics similar to those of immunotherapy-sensitive GC. However, *H. pylori*–positive patients with DNA mismatch repair–deficient (dMMR)/microsatellite instability–high (MSI-H) colorectal adenocarcinoma and esophageal squamous cell carcinoma (ESCC) had shorter irPFS compared with *H. pylori*–negative patients (16.13 months versus not reached, $p = 0.042$, HR 2.26, 95% CI 1.13–4.50, $p = 0.021$ and 5.57 months versus 6.97 months, $p = 0.029$, HR 1.59, 95% CI 1.14–2.23, $p = 0.006$, respectively). The difference in irOS between *H. pylori*–positive and –negative patients had the same trend as that between dMMR/MSI-H colorectal adenocarcinoma and ESCC patients. We also identified a trend of shorter irPFS and irOS in *H. pylori*–positive liver cancer and pancreatic cancer patients. In summary, our findings supported that *H. pylori* infection is a beneficial factor for GC immunotherapy by shaping hot tumor microenvironments. However, in dMMR/MSI-H colorectal adenocarcinoma and ESCC patients, *H. pylori* adversely affects the efficacy of immunotherapy.

INTRODUCTION

Helicobacter pylori is a widely prevalent bacterial infection of the gastric mucosa, affecting over 40% of the global population.[1] The World Health Organization recognized *H. pylori* as a group 1 carcinogen for gastric cancer (GC).[2–4] *H. pylori* contributes to gastric carcinogenesis via various mechanisms, including the injection of two cytotoxins, VacA and CagA, into host cells.[5,6] Recent epidemiological surveys and laboratory research revealed that *H. pylori* infection is involved in promoting the development of colorectal adenocarcinoma.[7] However, the pathogenic mechanisms of *H. pylori* infection on GC and colorectal adenocarcinoma are different.[7–9] The relationships between *H. pylori* and the risk of esophageal cancer were contradictory in different studies.[10,11] Previous randomized controlled trials, including our group's study, have demonstrated that *H. pylori* eradication can decrease the risk of GC.[12–14] Our group reported a prospective randomized intervention trial with 22 years of follow-up. We observed a decreased mortality rate due to colorectal adenocarcinoma in the group treated for *H. pylori* compared with the placebo group.[15] Current evidence suggests that *H. pylori* infection may have wide-ranging effects on gastrointestinal (GI) cancers, with potentially different outcomes depending on the specific cancer type.

Immunotherapy has changed the treatment landscape since its initial approval for microsatellite instability–high (MSI-H) tumors in 2015. Recent clinical trials have demonstrated its remarkable efficacy in treating GI cancers, prompting a paradigm shift in treatment guidelines.[16,17] Specifically, studies evaluating anti-programmed cell death protein 1/PD ligand 1 (PD-1/PD-L1) therapy have revealed long-lasting responses ranging from 30% to 50% in advanced GC and up to 60% in DNA mismatch repair–deficient (dMMR)/MSI-H colorectal adenocarcinoma.[18–21] The efficacy of immunotherapy may differ in different types of cancer, emphasizing the importance of personalized approaches guided by robust clinical evidence.[22] To overcome tumor heterogeneity and identify patients who could benefit from anti-PD-1/PD-L1 therapy, several biomarkers, including PD-L1 combined positive score (CPS), Epstein-Barr virus (EBV) infection, dMMR or MSI-H status, and other biomarkers, have been developed for stratifying GI cancers.[19,23–26] Our previous research emphasized the significant association between tumor microenvironment (TME) and clinical efficacy of immunotherapy in patients with GC.[27] Several factors can remodel TME, thereby affecting anti-PD-1/PD-L1 therapy. Patients with EBV-positive GC display a higher presence of CD8$^+$ cytotoxic T cells, mature dendritic cells, and PD-L1 expression within the TME, which may be favorable for immunotherapy.[26,28]

Previous studies have reported that *H. pylori*–positive GC patients have a higher expression of PD-L1 compared with *H. pylori*–negative GC patients.[29] Based on emerging evidence, we hypothesized that *H. pylori* infection may influence the treatment outcome of immunotherapy for GI cancers. To investigate this hypothesis, in this study, we aimed to examine the association between *H. pylori* infection status and the treatment efficacy of anti-PD-1/PD-L1 therapy. In addition, we compared the immune and transcriptomic characteristics between *H. pylori*–positive and *H. pylori*–negative GC patients.

RESULTS

Patients

In total, 10,122 patients performed ^{13}C-urea breath tests between September 22, 2016 and April 19, 2023 in the Department of Gastrointestinal Oncology and Early Drug Development Center at Peking University Cancer Hospital & Institute. No allergic reactions were observed during the tests. After excluding 616 non-cancer patients and 163 patients with ≥2 primary tumors, 9,343 patients were included in the study. The included patients were further classified into 2 subgroups: 8,746 patients with GI cancers and 597 with non-GI tumors (Figure S1). Among them, we identified 2,460 patients who had received anti-PD-1/PD-L1 therapy. Details on the numbers of patients in each subgroup are presented in Figure S2A. Three subgroups with the highest rate of *H. pylori* infection were GC (44.19%), esophageal cancer (42.35%), and colorectal adenocarcinoma (33.24%), suggesting a prevalence of *H. pylori* infection in GI cancers (Figure S2B).

H. pylori infection and immunotherapy for GC

We analyzed the data of 2,714 patients with gastric adenocarcinoma. Table S1 summarizes the clinicopathological characteristics of these patients. We found a higher proportion of *H. pylori*–positive patients exhibiting PD-L1 CPS ≥5. Because EBV-positive or dMMR/MSI-H GC were previously demonstrated to be sensitive for immunotherapy for active TME,[30] we included 636 EBV-negative microsatellite stable (MSS) GC patients receiving immunotherapy for our main hypothesis (Table 1). The detailed chemotherapy protocols for immunotherapy combined with chemotherapy are presented in Table S2. The inclusion and exclusion procedures are provided in Figure 1A.

Table 1. Baseline characteristics of 636 gastric adenocarcinoma patients

Characteristic	Total (N = 636)	*H. pylori* negative (N = 323)	*H. pylori* positive (N = 313)	p
Age at diagnosis, years				0.087
<65	353 (55.5)	190 (58.8)	163 (52.1)	
≥65	283 (44.5)	133 (41.2)	150 (47.9)	
Gender				0.265
Male	442 (69.5)	218 (67.5)	224 (71.6)	
Female	194 (30.5)	105 (32.5)	89 (28.4)	
Stage at diagnosis				0.798
I	0 (0.0)	0 (0.0)	0 (0.0)	
II	3 (0.5)	1 (0.3)	2 (0.6)	
III	39 (6.1)	22 (6.8)	17 (5.4)	
IV	585 (92.0)	296 (91.6)	289 (92.3)	
Unknown	9 (1.4)	4 (1.2)	5 (1.6)	
Tumor differentiation				0.356
High	4 (0.6)	3 (0.9)	1 (0.3)	
Moderate	146 (23.0)	66 (20.4)	80 (25.6)	
Poor	451 (70.9)	235 (72.8)	216 (69.0)	
Unknown	35 (5.5)	19 (5.9)	16 (5.1)	
Lauren classification				0.023
Intestinal type	253 (39.8)	117 (36.2)	136 (43.5)	
Mixed type	146 (23.0)	67 (20.7)	79 (25.2)	
Diffused type	205 (32.2)	119 (36.8)	86 (27.5)	
Unknown	32 (5.0)	20 (6.2)	12 (3.8)	
Location				0.117
GEJ	181 (28.5)	83 (25.7)	98 (31.3)	
Non-GEJ	455 (71.5)	240 (74.3)	215 (68.7)	
EGFR expression				0.857
0	55 (8.6)	24 (7.4)	31 (9.9)	
1	138 (21.7)	72 (22.3)	66 (21.1)	
2	222 (34.9)	115 (35.6)	107 (34.2)	
3	120 (18.9)	61 (18.9)	59 (18.8)	
Unknown	101 (15.9)	51 (15.8)	50 (16.0)	
HER2 expression				<0.001
Positive	127 (20.0)	45 (13.9)	82 (26.2)	
Negative	483 (75.9)	262 (81.1)	221 (70.6)	
Unknown	26 (4.1)	16 (5.0)	10 (3.2)	
PD-L1 CPS				0.842
CPS<1	231 (36.3)	121 (37.5)	110 (35.1)	
1≤CPS<5	97 (15.3)	49 (15.2)	48 (15.3)	
5≤CPS<10	81 (12.7)	42 (13.0)	39 (12.5)	
CPS>10	153 (24.1)	78 (24.1)	75 (24.0)	
Unknown	74 (11.6)	33 (10.2)	41 (13.1)	
Line of therapy				0.006
1	409 (64.3)	191 (59.1)	218 (69.6)	
≥2	227 (35.7)	132 (40.9)	95 (30.4)	
Type of anti-PD-1/PD-L1 therapy				0.173
Immunotherapy	116 (18.2)	64 (19.8)	52 (16.6)	
Immunotherapy combined with chemotherapy	290 (45.6)	154 (47.7)	136 (43.5)	
Immunotherapy combined with target therapy	112 (17.6)	55 (17.0)	57 (18.2)	
Immunotherapy combined with chemotherapy and target therapy	118 (18.6)	50 (15.5)	68 (21.7)	

GEJ, gastroesophageal junction; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2.

The median follow-ups of irPFS and irOS were 9.80 months (interquartile range 3.20–20.27) and 18.77 months (interquartile range 7.83–28.33), respectively. The presence of *H. pylori* infection in GC patients was significantly associated with longer irPFS (p < 0.001, HR, 0.76, 95% CI 0.62–0.95, p = 0.015; Figure 2A; Tables 2 and S3). The median irOS of *H. pylori*–positive patients was 4 months longer than that of *H. pylori*–negative patients (Figure 2B; Tables 2 and S3). After multivariable adjustment, *H. pylori* infection was associated with a higher objective response rate (ORR) (odds ratio 0.45, 95% CI 0.30–0.69, p < 0.001; Table 3).

The *H. pylori*–positive groups had more patients receiving first-line immunotherapy, potentially leading to longer irPFS and irOS. Therefore, we performed subgroup analyses stratified by the lines of immunotherapy (Figure S3A). In the subgroup of patients receiving first- or second-line and above immunotherapy, we observed a trend toward longer irPFS among *H. pylori*–positive patients. However, no statistical difference in irOS was observed between *H. pylori*–positive and –negative GC patients (Figures S3B and S3C).

Results from the CheckMate-649 clinical trial revealed that patients with GC who exhibit lower PD-L1 CPS scores may derive limited benefits from anti-PD-1/PD-L1 immunotherapy.[19] Therefore, we stratified the patients based on PD-L1 CPS of 1 or 5 in the subgroup analysis. We observed that *H. pylori*–positive patients had longer irPFS (p = 0.006, HR, 0.62, 95% CI 0.44–0.87, p = 0.094) and irOS (p = 0.010, HR 0.76, 95% CI 0.52–1.11, p = 0.160) than *H. pylori*–negative patients in the PD-L1 CPS <1 subgroup (Figures S4A and S4B; Table S4). Identical trends were observed in the PD-L1 CPS <5 subgroup (Figures S5A and S5B). Our analysis of the PD-L1 CPS ≥1 or ≥5 subgroup revealed no significant differences in both irPFS and irOS between *H. pylori*–positive and –negative patients. (Figures S4C and S5C).

Subgroup analyses were also conducted for $HER2^+$ and $HER2^-$ patients (Figure S6). In the $HER2^-$ subgroup, *H. pylori*–positive patients had significantly longer irPFS than *H. pylori*–negative patients (p = 0.026). Similar trends were observed in the subgroup analyses of EBV-positive GC and dMMR/MSI-H GC patients (Figures S7 and S8).

H. pylori infection and immunotherapy for dMMR/MSI-H colorectal adenocarcinoma and esophageal squamous cell carcinoma (ESCC)

dMMR/MSI-H colorectal adenocarcinoma was approved for anti-PD-1/PD-L1-based immunotherapy owing to its sensitivity to immunotherapy.[31] Because adenocarcinoma was the main type of colorectal adenocarcinoma, we included 112 dMMR/MSI-H colorectal adenocarcinoma patients to test our main hypothesis (Figure 1B; Table S5). The median follow-ups for irPFS and irOS were 21.27 (interquartile range 7.57–36.83) and 31.07 months (interquartile range

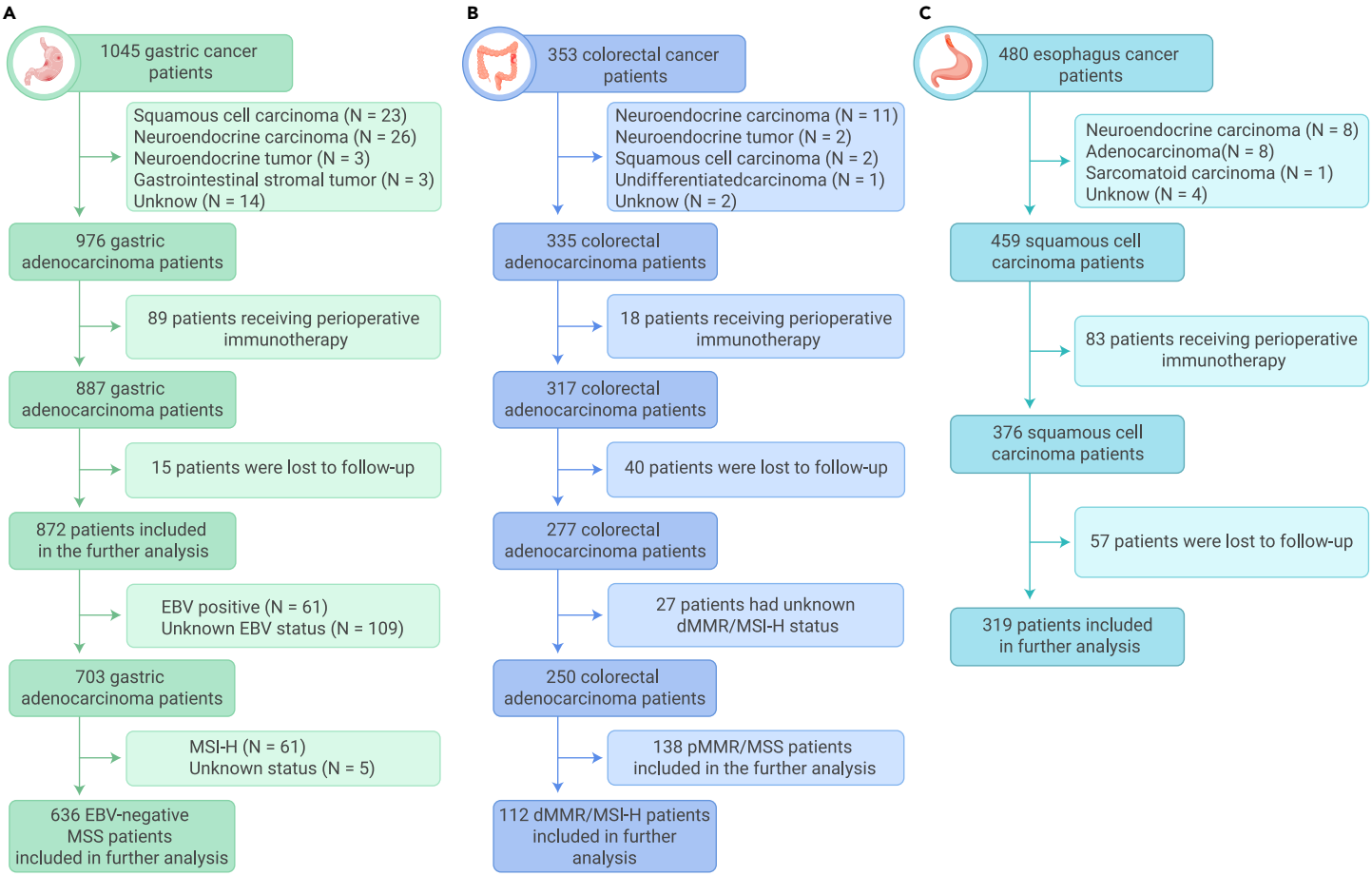

Figure 1. The inclusion and exclusion procedures for main hypotheses (A–C) The inclusion and exclusion steps of EBV-negative MSS GC patients, dMMR/MSI-H colorectal adenocarcinoma patients, and esophageal cancer patients, respectively.

16.97–52.27), respectively. Our analyses revealed that *H. pylori*–positive dMMR/MSI-H colorectal adenocarcinoma patients had significantly shorter median irPFS compared with their *H. pylori*–negative counterparts (p = 0.042, HR 2.26, 95% CI 1.13–4.50, p = 0.021; Figure 3A; Tables 2 and S6). Furthermore, the negative effect of *H. pylori* on anti-PD-1/PD-L1 immunotherapy was evident in our analysis on irOS (p = 0.001, HR 3.34, 95% CI 1.44–7.72, p = 0.005; Figure 3B). We also conducted exploratory analyses for pMMR/MSS colorectal adenocarcinoma patients and observed a trend toward shorter irPFS in the *H. pylori*–positive group (Figure S9).

We included 319 ESCC patients in the statistical analyses for our main hypotheses (Figure 1C; Table S7). The median follow-ups for irPFS and irOS were 6.30 (interquartile range 2.67–19.47) and 17.80 months (interquartile range 8.40–29.37), respectively. The irPFS of *H. pylori*–positive ESCC patients (5.57 months) was shorter than that of *H. pylori*–negative patients (6.97 months) (p = 0.029, HR 1.59, 95% CI 1.14–2.23, p = 0.006; Figure 4A; Tables 2 and S8). Compared with *H. pylori*–negative patients, *H. pylori*–positive patients showed shorter irOS, although the difference was not significant (17.27 months versus 21.63 months, p = 0.208, HR 1.48, 95% CI 1.05–2.10, p = 0.027; Figure 4B).

Exploratory analyses for *H. pylori* infection and immunotherapy

We conducted exploratory analyses for patients with tumors that were not GC, colorectal adenocarcinoma, or ESCC. We first distinguished patients with stromal tumors, neuroendocrine carcinoma, and neuroendocrine tumors from those having other tumors because of their unique molecular and clinicopathological characteristics.[32,33] No difference was observed in the irPFS and irOS between *H. pylori*–positive and *H. pylori*–negative patients with stromal tumor (N = 3), neuroendocrine carcinoma (N = 88), or neuroendocrine tumors (N = 21) (Figure S10). Based on the primary site of malignancy, the patients were categorized into liver cancer, pancreatic cancer, cholangiocarcinoma, gallbladder carcinoma, small bowel cancer, ampullary cancer, and non-GI cancer subgroups. Although differences in median irPFS and irOS were observed between *H. pylori*–positive and *H. pylori*–negative patients in certain tumor types, these findings were not statistically significant (Figures S11–S13).

In non-GI tumor patients, *H. pylori* positivity was associated with a decreased median irPFS and irOS compared with *H. pylori* negativity (Figures S14A and S14B). Prior studies have demonstrated that *H. pylori* seropositivity was significantly correlated with a decreased median irPFS in patients with non-small cell lung cancer who received anti-PD-1 therapy.[34] In our cohort, we observed that all 8 *H. pylori*–positive patients with lung cancer had disease progression within 12 months of immunotherapy, whereas 5 of 16 *H. pylori*–negative patients had SD (Figures S14C and S14D). In general, the results should be interpreted cautiously owing to the limited number of patients.

TME of *H. pylori*–positive GC

We used mIHC to visualize the TME of 170 patients with gastric adenocarcinomas. Of them, 79 (46.5%) were *H. pylori* positive. We initially evaluated the density of immune checkpoint–positive cells (Figures 5A, 5B, and S15A) and observed significantly higher densities of PD-L1$^+$ and PD-1$^+$ T cells in *H. pylori*–positive GC patients, which was consistent with the results of previous meta-analyses.[29] We further examined the functional subtype of the cells. Although the densities of CD4$^+$ T and regulatory T cells (Tregs) did not differ between *H. pylori*–positive and *H. pylori*–negative GC patients, a significant enrichment of CD4$^+$PD-L1$^+$ and CD4$^+$CTLA-4$^+$PD-L1$^+$ T cells was observed in *H. pylori*–positive GC patients (Figures 5C, 5D, and S15B). In the TME of *H. pylori*–positive GC patients, we observed a higher density of nonexhausted CD8$^+$ T cells (CD8$^+$LAG-3$^-$PD-1$^-$TIM-3$^-$), whereas the density of exhausted CD8$^+$ T cells (CD8$^+$PD-1$^+$) did not change between the *H. pylori*–positive and *H. pylori*–negative groups (Figures 5E, 5F, and S15C). In addition, the density

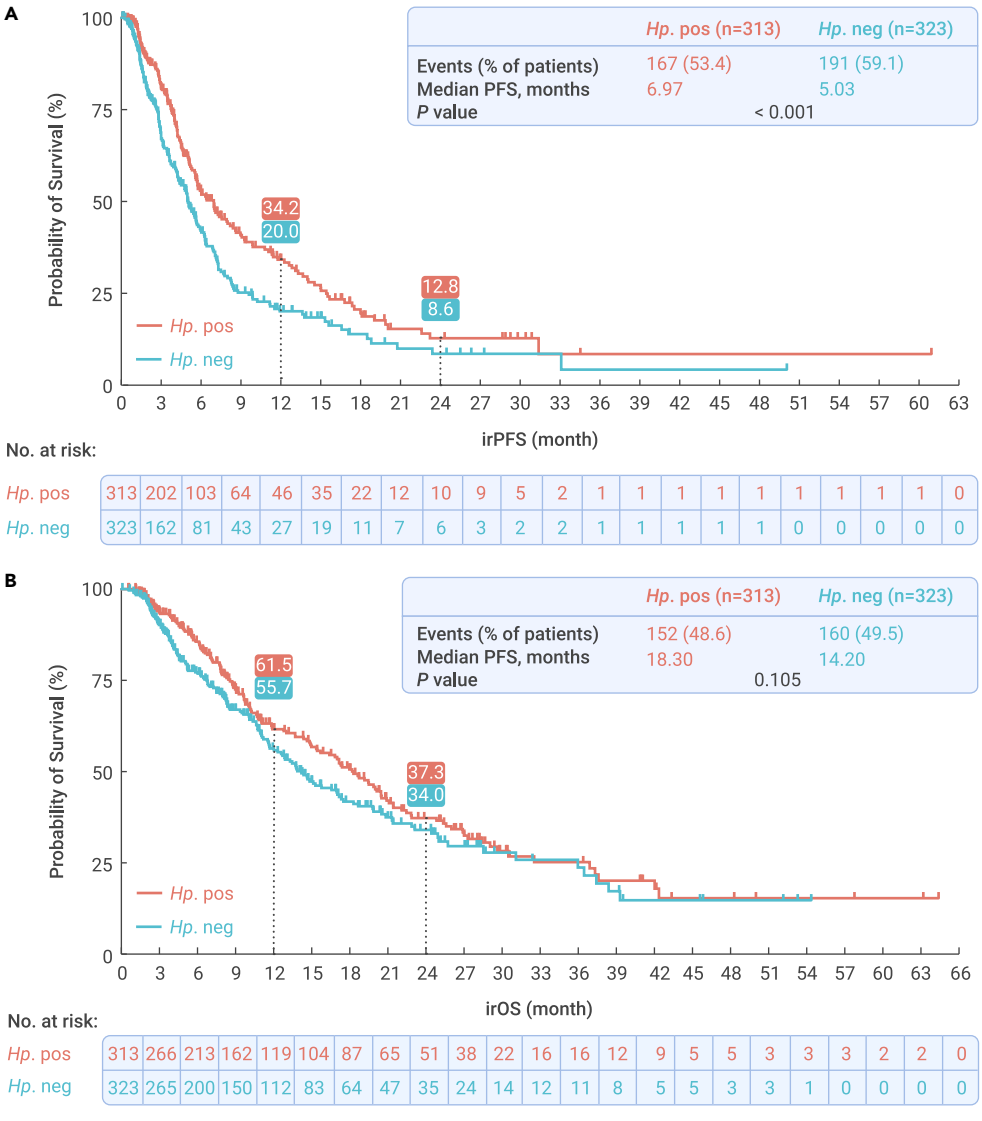

Figure 2. Survival curves of EBV-negative MSS gastric adenocarcinoma patients (A and B) The survival analysis of irPFS and irOS of EBV-negative MSS gastric adenocarcinoma patients, respectively. These patients were grouped by their *H. pylori* infection status. Survival analyses were conducted by log rank test. p values are two sided.

of macrophages and B cells did not differ between the two groups (Figures S15D and S15E).

We conducted RNA sequencing on 43 gastric adenocarcinoma samples. *H. pylori* infection was positive in 51.2% of cases. We analyzed the expression of *PDCD1* (which codes for PD-1 protein) and *CD274* (which codes for PD-L1 protein) and observed significantly higher expression levels in *H. pylori*–positive samples compared with *H. pylori*–negative samples (Figures 5G and 5H). To investigate mechanisms underlying the effect of *H. pylori* infection on GC, we used GSVA to quantitatively measure pathway activities based on the gene signature used in the CheckMate-649 biomarker analyses.[35] The results showed a significant increase in the proliferation-related score and a decrease in the stroma-related score in the *H. pylori*–positive group (Figure 5I). Notably, patients with higher proliferation-related scores or lower stroma-related scores benefited more from nivolumab plus ipilimumab treatment in the CheckMate-649 clinical trial.[35]

DISCUSSION

To the best of our knowledge, this is the largest retrospective study analyzing the relationship between *H. pylori* infection and immunotherapy of multiple cancer types. Our findings revealed a previously unknown protective effect of *H. pylori* infection on immunotherapy for GC. *H. pylori*–positive GC had higher densities of PD-L1$^+$ immune cells and nonexhausted CD8$^+$ T cells in TME, making it a "hot" tumor. Moreover, we found altered characteristics of proliferation and stroma in *H. pylori*–positive GC that, altogether, enhance its sensitivity to immunotherapy. Furthermore, *H. pylori* infection had an adverse impact on the treatment response of immunotherapy in dMMR/MSI-H colorectal adenocarcinoma and ESCC patients. We observed a trend of shorter survival in *H. pylori*–positive liver cancer and pancreatic cancer patients, suggesting that the effect of *H. pylori* infection on GI cancers is comprehensive and complex. Therefore, our research reveals the contradictory effects of *H. pylori* on different types of cancer, highlighting the importance of performing the *H. pylori* test as a part of immunotherapy for GI cancer.

In the present study, we observed a significantly longer irPFS in *H. pylori*–positive GC patients, which may be attributed to the unique characteristics of its TME. TME can be classified as (1) PD-L1$^-$, tumor-infiltrating lymphocyte (TIL)–negative ("cold" tumors); (2) PD-L1$^+$, TIL$^+$ (hot tumors); (3) PD-L1$^-$, TIL$^+$; and (d) PD-L1$^+$, TIL$^-$ tumor subtypes.[36] *H. pylori*–positive GCs were reportedly associated with higher PD-L1 expression, which was consistent with the results of our study.[29] Furthermore, we revealed that *H. pylori*–positive GCs exhibited higher densities of PD-L1$^+$ and nonexhausted CD8$^+$ T cells compared with *H. pylori*–negative GC. This finding suggests that *H. pylori*–positive GC has a hot tumor phenotype, characterized by heightened immunogenicity and sensitivity to immunotherapeutic interventions.[37] The elevated expression of PD-L1 in *H. pylori*–positive GC observed in our cohort was consistent with previous findings,[29] and future studies should incorporate *in vivo* and *in vitro* experiments to explore the causality behind this association.

Except for the upregulated expression of PD-L1, *H. pylori* infection has been associated with DNA damage and its interference with DNA repair capabilities of the TME.[38] *H. pylori* infection was also involved in the modulation of immune cell functions in TME.[39,40] Furthermore, *H. pylori* has been reported to significantly inhibit the colonization of other bacteria in the stomach of non-GC patients, resulting in reduced gastric microbiota diversity. However, the impact of *H. pylori* on microbiota in the stomach of GC patients remains unclear.[41] Given the extensive influence of *H. pylori* on the TME of GC, investigating which altered factors caused by *H. pylori* may affect the efficacy of immunotherapy is necessary.

CheckMate-649 demonstrated that compared with chemotherapy, nivolumab plus chemotherapy could not significantly prolong irPFS of GC patients with PD-L1 CPS <1 and <5.[19] Our study provided evidence that *H. pylori* infection status could aid in the stratification of patients with PD-L1 CPS <1 or <5 who had longer immunotherapy-related survival. Our findings highlighted that the favorable effect of *H. pylori* infection persisted even in the absence of PD-L1 expression, which may be attributable to the remodeling effect of *H. pylori* on the TME of GC.[42] The higher density of nonexhausted CD8$^+$ T cells in *H. pylori*–positive GC indicated its potential antitumor effect.[43] Hence, future studies should prioritize the examination of more functional subtypes of CD8$^+$ T cells, such as CD103$^+$CD8$^+$ T cells, which are assumed to be predictive of the response to PD-L1 blockade.[44] In addition, our findings indicated that *H. pylori*–positive GC had a higher proliferation-related and lower stroma-related scores; these results aligned with the gene signatures of patients

Table 2. Association of *H. pylori* status with survival in multivariable Cox regression models in GC, dMMR/MSI-H colorectal adenocarcinoma, and ESCC patients

	irPFS				irOS		
	No. cases	No. events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. events	Univariate HR (95% CI)	Multivariate HR (95% CI)
GC[a]							
H. pylori status							
Negative	323	191	1 (reference)	1 (reference)	160	1 (reference)	1 (reference)
Positive	313	167	0.70 (0.57–0.86)	0.76 (0.62–0.95)	152	0.83 (0.67–1.04)	0.98 (0.78–1.23)
p value			0.001	0.015		0.11	0.84
dMMR/MSI-H colorectal adenocarcinoma[b]							
H. pylori status							
Negative	81	21	1 (reference)	1 (reference)	11	1 (reference)	1 (reference)
Positive	31	14	1.99 (1.01–3.92)	2.26 (1.13–4.50)	12	2.82 (1.24–6.42)	3.34 (1.44–7.72)
p value			0.046	0.021		0.013	0.005
ESCC[c]							
H. pylori status							
Negative	194	91	1 (reference)	1 (reference)	84	1 (reference)	1 (reference)
Positive	125	64	1.43 (1.04–1.98)	1.59 (1.14–2.23)	56	1.24 (0.89–1.74)	1.48 (1.05–2.10)
p value			0.030	0.006		0.21	0.027

[a]The multivariable, stage (stage II versus III versus IV)-stratified Cox regression model initially included tumor location (GEJ versus non-GEJ), tumor differentiation (high versus moderate versus poor), Lauren classification (intestinal type versus diffused type versus mixed type), HER2 expression (positive versus negative), PD-L1 expression (CPS<1 versus 1–5 versus 5–10 versus >10 versus unknown), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.
[b]The multivariable, stage (stage II versus III versus IV)-stratified Cox regression model initially included tumor differentiation (high versus moderate versus poor), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.
[c]The multivariable, stage (stage II versus III versus IV)-stratified Cox regression model initially included tumor differentiation (high versus moderate versus poor), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.

who were responsive to immunotherapy in the CheckMate-649 study.[35] The potential of proliferation-associated gene signatures to predict the outcome of immune checkpoint inhibitors was also demonstrated in an independent lung cancer cohort.[45] The stroma-related score was constructed based on the gene markers for fibroblasts.[46] Although the functional roles of various cancer-associated fibroblast subtypes remain largely unknown, many studies have reported their clinical value as prognostic factors for immunotherapy.[47] In general, our study not only provides evidence regarding the positive effect of *H. pylori* infection on immunotherapy for GC but also elucidates the potential underlying mechanisms.

Regarding dMMR/MSI-H colorectal adenocarcinoma, this is the first study to reveal the adverse effect of *H. pylori* on immunotherapy. These tumors had a "hot" TME with a high abundance of immune cell infiltrates, particularly $CD8^+$ TILs, T helper 1 cells, $CD4^+$ TILs, and macrophages, making them good candidates for immunotherapy.[48,49] However, there remained 30%–45% of dMMR/MSI-H colorectal adenocarcinoma patients who could not benefit from immunotherapy.[24,49] Our findings suggest that *H. pylori* infection status may serve as a powerful biomarker to identify dMMR/MSI-H colorectal adenocarcinoma patients who may not benefit from immunotherapy. Further prospective research is required to determine whether eradicating *H. pylori* can improve the efficacy of immunotherapy in these dMMR/MSI-H colorectal adenocarcinoma patients. Previous studies have demonstrated that *H. pylori* infection affects the gut microbiome of healthy individuals.[50] However, the effect of *H. pylori* infection on the gut microbiome of colorectal adenocarcinoma patients has yet to be fully understood. Further investigations are needed to establish the causal relationship among *H. pylori* infection, alterations in the gut microbiome, and immunotherapy response in colorectal adenocarcinoma patients.

In this study, we also found that *H. pylori* infection was a negative factor in immunotherapy for ESCC. Similarly, Oster et al. have demonstrated that *H. pylori* infection was related with adverse effects to immunotherapy for lung cancer.[34] After integrating the data of non-GI cancers in our cohort, we observed a shorter irPFS in the *H. pylori*–positive group. This result indicates that *H. pylori* infection has a wide-ranging effect not only on GI cancers but also on non-GI cancers. To explain the possible mechanism of *H. pylori* infection on cancers, Oster et al. constructed subcutaneous xenograft tumor models and proposed that *H. pylori* infection dampened innate immune responses to tumors, such as the defects in cross-presentation activities of dendritic cells in the spleen.[34] In general, the mechanism by which *H. pylori* affects immunotherapy should be investigated further. Our study highlights the importance of *H. pylori* infection test in cancer immunotherapy.

This study had some limitations. First, this was a single-center study. However, because the study used data from a first-class cancer hospital in China, the patients included in the study come from all over the country, thereby increasing the representativeness of this study. Second, the nature of a retrospective study may limit the implication of our results compared with those of prospective clinical trials. To solve this problem, we did thorough analyses including multiple Cox model adjustments and subgroup analysis to obtain solid results. Finally, the mechanism by which *H. pylori* adversely affects immunotherapy efficacy for dMMR/MSI-H colorectal adenocarcinoma and ESCC remains unclear; however, we presented possible mechanisms for the positive effect of *H. pylori* infection on immunotherapy for GC.

In conclusion, *H. pylori* infection shaped the "hot" TME of GC, thereby becoming a favorable prognostic factor for immunotherapy in GC patients. However, *H. pylori* infection is unfavorable for immunotherapy in patients with dMMR/MSI-H colorectal adenocarcinoma, ESCC, and other GI cancers.

Table 3. Ordinal logistic regression analysis to assess the association of *H. pylori* infection with ORR in GC, dMMR/MSI-H colorectal adenocarcinoma, and ESCC patients treated with immune checkpoint inhibitors

	Objective response	
	Univariable OR (95% CI)	Multivariable OR (95% CI)
GC[a]		
H. pylori status		
Negative	1 (reference)	1 (reference)
Positive	0.42 (0.29–0.62)	0.45 (0.30–0.69)
p value	<0.001	<0.001
dMMR/MSI-H colorectal adenocarcinoma[b]		
H. pylori status		
Negative	1 (reference)	1 (reference)
Positive	1.58 (0.60–4.17)	1.49 (0.53–4.19)
p value	0.36	0.45
ESCC[c]		
H. pylori status		
Negative	1 (reference)	1 (reference)
Positive	1.46 (0.90–2.38)	1.63 (0.99–2.70)
p value	0.13	0.057

OR, odds ratio.
[a]The multivariable ordinal logistic regression model initially included stage (stage II versus III versus IV), tumor location (GEJ versus non-GEJ), tumor differentiation (high versus moderate versus poor), Lauren classification (intestinal type versus diffused type versus mixed type), HER2 expression (positive versus negative), PD-L1 expression (CPS<1 versus 1–5 versus 5–10 versus >10 versus unknown), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.
[b]The multivariable ordinal logistic regression model initially included stage (stage II versus III versus IV), tumor differentiation (high versus moderate versus poor), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.
[c]The multivariable ordinal logistic regression model initially included stage (stage II versus III versus IV), tumor differentiation (high versus moderate versus poor), lines of therapy (1 versus ≥2), and types of therapy (immunotherapy versus immunotherapy combined with chemotherapy versus immunotherapy combined with target therapy versus immunotherapy combined with chemotherapy and target therapy). A backward elimination with a threshold of p = 0.05 was used to select variables in the final models.

MATERIALS AND METHODS

Study oversight

We conducted a retrospective study encompassing multiple cancer types at the Peking University Cancer Hospital & Institute. Informed consent forms were obtained from patients or their legal guardians in advance of participation in the study. The study was approved by the ethics committee of Peking University Cancer Hospital & Institute (2020KT08). The research study was conceived and designed by the corresponding author, and it received financial support from the government and nonprofit organizations. The data collection and analysis were carried out by study physicians specializing in oncology.

Patients

Between September 22, 2016 and April 19, 2023, we conducted ^{13}C-urea breath tests on eligible patients at the Department of Gastrointestinal Oncology and Early Drug Development Center of Peking University Cancer Hospital & Institute. To ensure the accuracy and reliability of our results, we excluded noncancer patients who were visiting the hospital for nondiagnostic reasons such as routine medical examinations and those with multiple primary tumors due to their therapeutic complexity.

Assessment

Detailed procedures of the ^{13}C-urea breath test performed in this study are described in the supplemental methods. We classified tumor responses into four categories according to the Response Evaluation Criteria in Solid Tumors (RECIST): complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). In this study, responders were defined as patients who achieved a CR or PR, whereas nonresponders were defined as those with SD or PD. Immune-related progression-free survival (irPFS) was defined as duration from the initiation of immunotherapy to the day when disease progression was observed, the patient died, or the end of follow-up, whichever came first. In addition, the time from initial immunotherapy to death or the end of follow-up, whichever came first, was referred to as immune-related overall survival (irOS). The last follow-up date of the patients in our study was May 16, 2023.

Multiplex immunohistochemistry

To analyze the composition of TME, we conducted multiplex immunohistochemistry (mIHC) to visualize PD-1, TIM-3, LAG-3, PD-L1, CTLA-4, CD8, CD4, FoxP3, CD68, CD163, and CD20. We used multispectral imaging to identify complex cell phenotypes, such as regulatory T cells and M2 macrophages, in each sample. Finally, we calculated the density of each cell subtype in our analysis. The detailed procedures for mIHC and multispectral image analysis are presented in the supplemental methods.

RNA-sequencing and bioinformatics analysis

The RNA-sequencing procedures are described in the supplemental methods. To analyze transcriptomic data, we performed the following steps: first, we normalized raw read counts by transforming them into transcripts per kilobase of exon model per million mapped reads. Second, we performed a log2 transformation on the expression matrix resulting from the previous normalization step. To identify differential gene expression between groups of samples, we used the R package limma (version 3.54.2). In addition, we performed gene set variation analysis (GSVA) to identify gene signatures associated with distinct biological processes[51]; this provided us with a quantitative measure of specific biological activity for each sample.

Statistical analysis

All of the statistical analyses were conducted using GraphPad Prism 9 software (GraphPad Software, San Diego, CA), IBM SPSS Statistics version 26 software (IBM Corp., Armonk, NY), and R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).[52] A two-sided p value <0.05 was considered statistically significant. The null hypothesis was that *H. pylori* infection has no effect on the efficacy of anti-PD-1/PD-L1 immunotherapy for GI cancers. The remaining statistical analyses were secondary; thus, the results should be interpreted with caution. For survival analysis, we used the Kaplan-Meier method and log rank test to compare the median irPFS and irOS between the *H. pylori*–positive and *H. pylori*–negative groups. To ensure reliable results and control for potential confounding variables, we used multivariable Cox proportional-hazard models to calculate the hazard ratio (HR) and 95% confidence interval (CI) for disease progression or mortality rates with respect to *H. pylori* infection status. The interaction was assessed using the Wald test for the cross-product of *H. pylori* infection status and PD-L1 expression. For further analysis, we estimated the odds ratios of *H. pylori* infection between responders and nonresponders using conditional logistic regression stratified on baseline clinicopathological characteristics. We also performed chi-square tests to assess the association between *H. pylori* infection and clinicopathological characteristics. In addition, we used unpaired Student's t test to compare the density of cell subtypes between *H. pylori*–positive and *H. pylori*–negative groups. Detailed descriptions of statistical analyses are provided in the supplemental methods.

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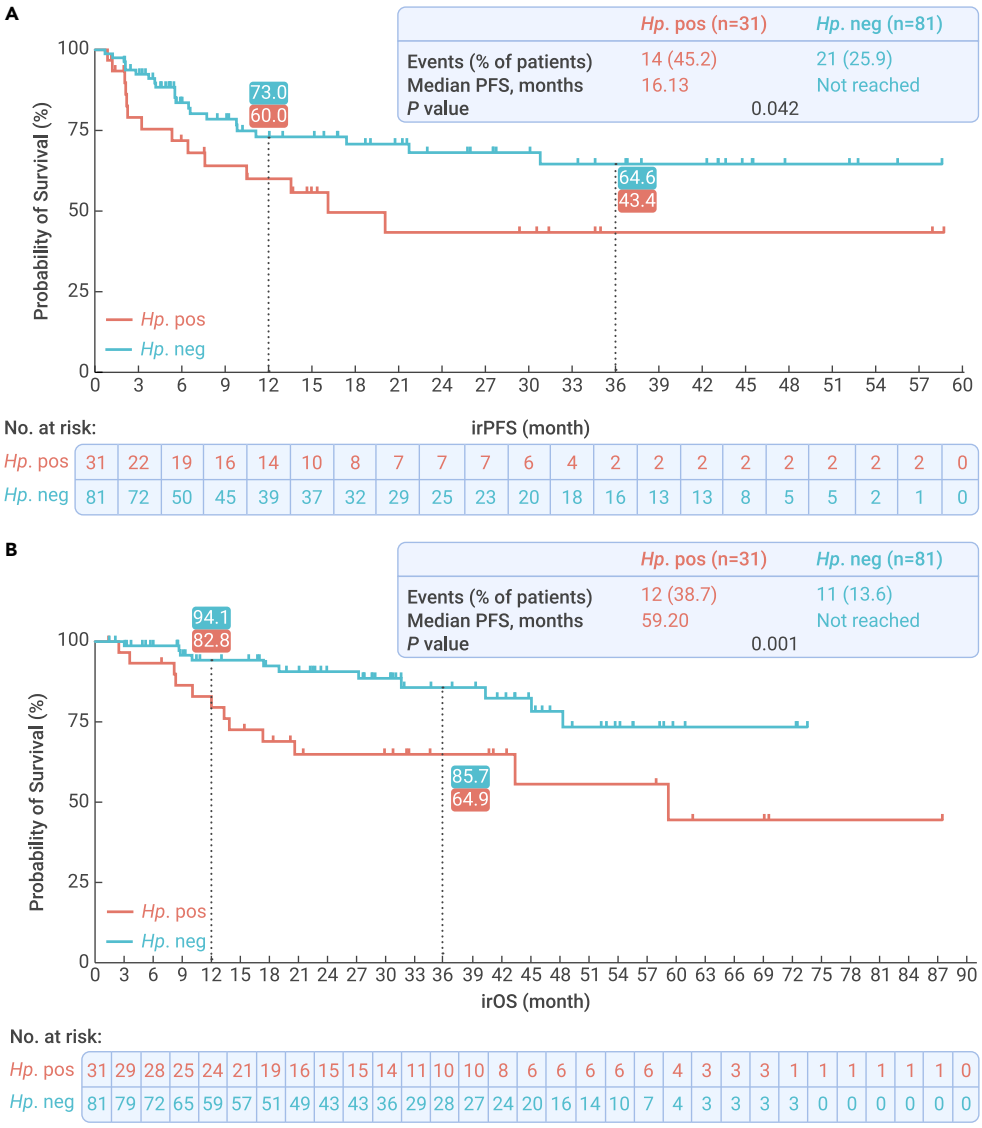

Figure 3. Survival curves of dMMR/MSI-H colorectal adenocarcinoma patients (A and B) The survival analysis of irPFS and irOS of dMMR/MSI-H colorectal adenocarcinoma, respectively. These patients were grouped by their *H. pylori* infection status. Survival analyses were conducted by log rank test. p values are two sided.

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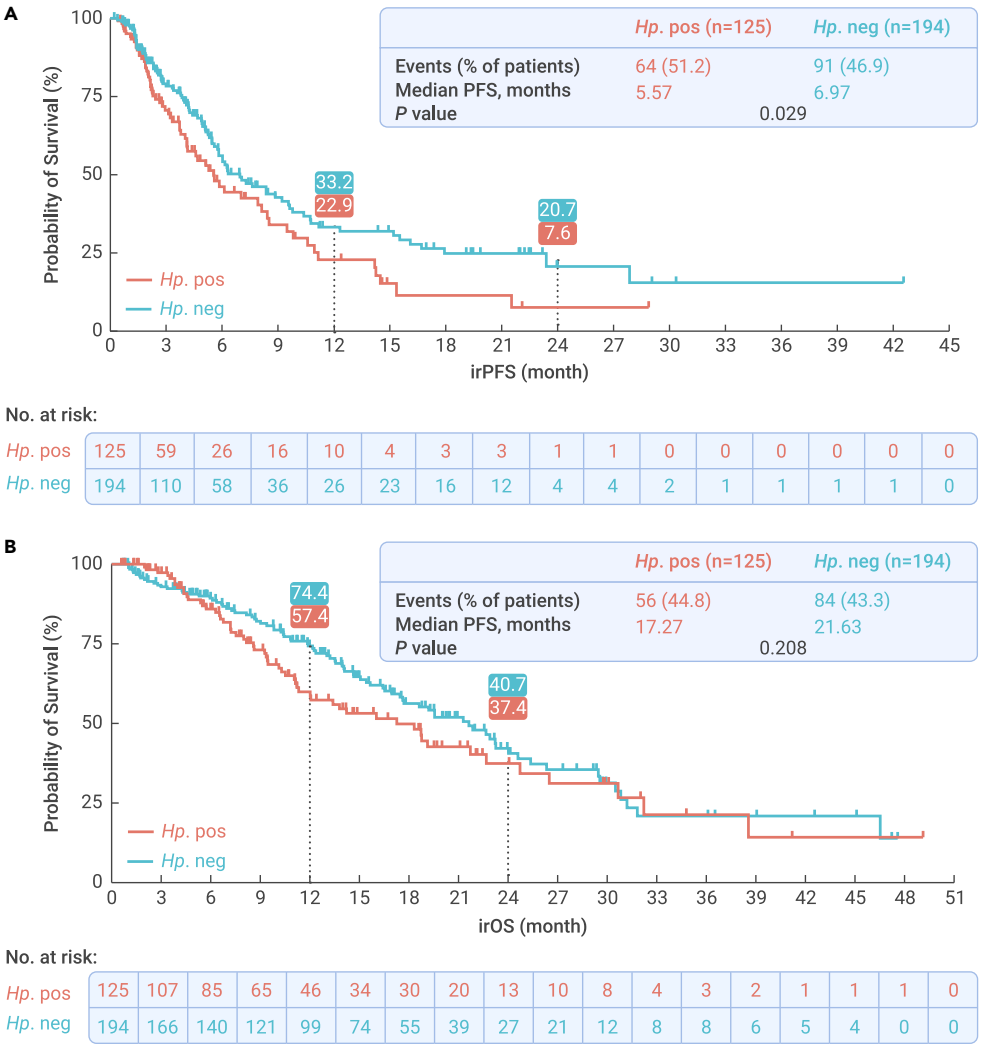

Figure 4. Survival curves of ESCC patients (A and B) The survival analysis of irPFS and irOS of ESCC, respectively. These patients were grouped by their *H. pylori* infection status. Survival analyses were conducted by log rank test. p values are two sided.

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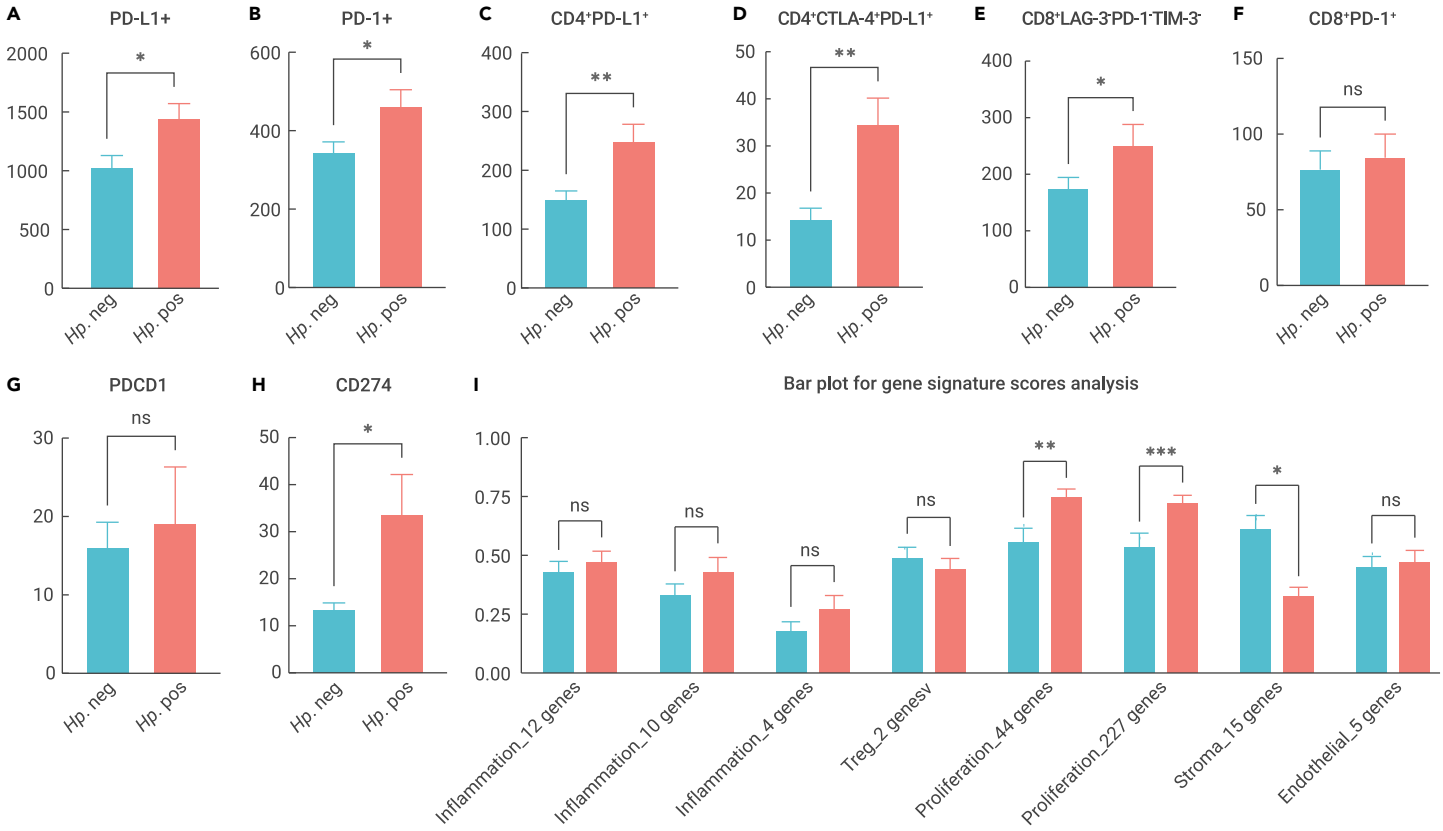

Figure 5. The molecular characteristics of the TME of *H. pylori*–positive and *H. pylori–negative* gastric adenocarcinoma. (A–F) Comparison of the density of immune cells between *H. pylori*–positive GC and *H. pylori*–negative GC by Student's t- test. (G and H) Comparison of PD-1 and PD-L1 expression level between *H. pylori*–positive and *H. pylori*–negative GC by limma package. (I) Comparison of gene signature scores between *H. pylori*–positive and *H. pylori*–negative GC by Student's t test. p values are two sided. *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.

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AUTHOR CONTRIBUTIONS

L.S. designed and supervised the study. Y.C., K.J., and Y.X. contributed to sample collection and the collection of patient clinical information. Y.S. and Y.H. contributed to the pathology review. X.W., Y.C., L.Z., Y.W., Z.W., and Z.L. contributed to data collection. K.J., Y.C., and Y.X. contributed to data processing, integrative analyses, writing the manuscript, and generating the figures and tables. J.L., X.Z., and L.S. revised the manuscript. All of the authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

It can be found online at https://doi.org/10.1016/j.xinn.2023.100561.

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