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ORIGINAL RESEARCH

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Helicobacter pylori serology is associated with worse overall survival in patients with melanoma treated with immune checkpoint inhibitors

Marion Tonneau[a,b], Alexis Nolin-Lapalme[a*], Suzanne Kazandjian[c*], Edouard Auclin[a], Justin Panasci[c], Myriam Benlaifaoui[a], Mayra Ponce[a], Afnan Al-Saleh[a], Wiam Belkaid[a], Sabrine Naimi[a], Catalin Mihalcioiu[c], Ian Watson[d], Mickael Bouin[e], Wilson Miller[f], Marie Hudson[f], Matthew K. Wong[g], Rossanna C. Pezo[g], Simon Turcotte[a,h], Karl Bélanger[a,i], Rahima Jamal[a,i], Paul Oster[j], Dominique Velin[j], Corentin Richard[a*], Meriem Messaoudene[a*], Arielle Elkrief[a*], and Bertrand Routy[a,i*]

[a]Axe Cancer, Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada; [b]Université de Médecine, Lille, France; [c]Department of Oncology, McGill University Health Center, QC, Canada; [d]Rosalind and Morris Goodman Cancer Institute, Montréal, QC, Canada; [e]Department of Gastroenterology, Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada; [f]Lady Davis Institute of the Jewish General Hospital, Montreal, QC, Canada; [g]Division of Medical Oncology, Sunnybrook Health Sciences Center, Odette Cancer Center, QC, Canada; [h]Department of Surgery, Centre Hospitalier de l'Université de Montréal, QC, Canada; [i]Division of Hemato-Oncology, Centre Hospitalier de l'Université de Montréal (CHUM)Montreal, QC, Canada; [j]Service of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland

ABSTRACT

The microbiome is now regarded as one of the hallmarks of cancer and several strategies to modify the gut microbiota to improve immune checkpoint inhibitor (ICI) activity are being evaluated in clinical trials. Preliminary data regarding the upper gastro-intestinal microbiota indicated that *Helicobacter pylori* seropositivity was associated with a negative prognosis in patients amenable to ICI. In 97 patients with advanced melanoma treated with ICI, we assessed the impact of *H. pylori* on outcomes and microbiome composition. We performed *H. pylori* serology and profiled the fecal microbiome with metagenomics sequencing. Among the 97 patients, 22% were *H. pylori* positive (Pos). *H. pylori* Pos patients had a significantly shorter overall survival ($p = .02$) compared to *H. pylori* negative (Neg) patients. In addition, objective response rate and progression-free survival were decreased in *H. pylori* Pos patients. Metagenomics sequencing did not reveal any difference in diversity indexes between the *H. pylori* groups. At the taxa level, *Eubacterium ventriosum, Mediterraneibacter (Ruminococcus) torques*, and *Dorea formicigenerans* were increased in the *H. pylori* Pos group, while *Alistipes finegoldii, Hungatella hathewayi* and *Blautia producta* were over-represented in the *H. pylori* Neg group. In a second independent cohort of patients with NSCLC, diversity indexes were similar in both groups and *Bacteroides xylanisolvens* was increased in *H. pylori* Neg patients. Our results demonstrated that the negative impact of *H. pylori* on outcomes seem to be independent from the fecal microbiome composition. These findings warrant further validation and development of therapeutic strategies to eradicate *H. pylori* in immuno-oncology arena.

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Introduction

Immune checkpoint inhibitors (ICIs) are now at the forefront of the management of patients with melanoma. Anti-PD-1 monotherapy or combination therapy with anti-CTLA-4 has led to unparalleled improvements in overall survival (OS) in patients with metastatic melanoma and represents the standard of care.[1] Despite improved outcomes with these new therapies, there is a need to develop robust biomarkers to adequately predict primary resistance, which occurs in 24–40% of patients.[2,3] Several intrinsic and extrinsic tumor factors such as PD-L1 expression, tumor – infiltrating lymphocytes, and neoantigens have been evaluated as biomarkers of ICI efficacy with moderate performances.[4] The gut microbiome has recently emerged as a biomarker of primary resistance and is now regarded as a hallmark of cancer.[5] Fecal microbiome profiling coupled with preclinical mouse experiments has revealed that beneficial bacteria are associated with ICI-response and immune-related side effect profile.[6–11] Moreover, several strategies to manipulate the microbiota including fecal microbiota transplantation have shown encouraging preliminary clinical results.[12–14]

While the focus has been on fecal commensal bacteria, the gastro-intestinal (GI) tract harbors various ecosystem subjected to different environmental conditions at different anatomical regions along the GI tract. *Helicobacter pylori* (*H. pylori*) colonizes the stomach mucosa and is present in over 50% of the world population. This bacterium was first discovered to cause gastric ulcer and MALT lymphoma.[15,16] Beyond the local effect in the stomach, recent evidence suggests that *H. pylori* has the potential to shift the macrophage polarization and could

CONTACT Bertrand Routy Bertrand.routy@umontreal.ca CRCHUM 900 Saint-Denis St, Montreal, Qc, H2X-0A9, Canada
*Authors contributed equally
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potentially alter systemic immune response.[17] Recently, Oster *et al.* demonstrated that neonatal infections with *H. pylori* in mice inhibited the ICI response through a shift in dendritic cell (DC) presentation to $CD8^+$ T cells independently of the fecal microbiome composition response.[18] The impact of *H. pylori* on the systemic immune response was further revealed in independent cohorts of patients with non-small cell lung cancer (NSCLC) and gastric cancer treated with ICIs where *H. pylori* seropositivity was associated with a shorter OS.[18,19]

Considering this observation in NSCLC, we sought to determine the impact of *H. pylori* on clinical outcomes of patients with advanced melanoma treated with ICI, and if the seropositivity of *H. pylori* influences the fecal microbiome composition.

Materials and methods

Patients

We conducted a multicenter retrospective cohort study of 97 patients with advanced melanoma. The study was conducted across four academic centers in Canada: Center Hospitalier de l'Université de Montréal (CHUM), the McGill University Health Center in Montreal (MUHC), the Jewish General Hospital in Montreal (JGH) and the Sunnybrook Health Sciences Center in Toronto. This study was approved by the institutional ethics committee (IRB: MP-02-2022-10115).

Inclusion criteria for this study were patients with a histologically proven diagnosis of advanced or unresectable melanoma treated with ICI therapy between 2013 and 2021. Patients with a diagnosis of uveal melanoma were excluded.

Tumor response was evaluated on CT scans using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[20] Immune related-adverse events (irAEs) were recorded according to CTCAE v5.[21] All patients were followed until death or until data lock on October 25, 2021.

H. pylori serology test

H. pylori serologic status (*H. pylori* IgG) was determined with validated commercial ELISA tests (*H. pylori* IgG ELISA Kit, Genesis Diagnostics). *H. pylori* IgG antibodies were quantified in the plasma of 97 patients, following the protocol for ELISA tests. The optical density (OD) at 450 nm was read in a microplate reader (Tecan, Infinite M200 Pro). The patients were stratified into *H. pylori* positive (Pos) or negative (Neg) groups according to their serology. Plasmas were collected as part of institutional biobank (Biobank Montreal Cancer Consortium 16.161 and TIME Study Sunnybrook).

Shotgun sequencing analysis

Fecal samples were obtained at the initiation of the ICI treatment for 44 of the 97 patients. We also examined fecal samples from patients with NSCLC (n = 28) (CRCHUM IRB 18.085) from a cohort published in Oster *et al.*[18] Feces were collected according to International Human Microbiome Standards (IHMS) guidelines (SOP 03 V1). Isolated DNA was analyzed using shotgun sequencing to investigate the microbial composition in fecal samples.[22] DNA was extracted following Suau *et al.*'s protocol.[23] The genetic material was subsequently sequenced using pyrosequencing. Resulting reads were then filtered using AlienTrimmer to both remove low quality reads as well as sequencing adapters.[24] The resulting cleaned data was further processed to remove human and other potential DNA contaminants. This was performed by removing any sequences matching to the human, *Bos taurus* and *Arabidopsis thaliana* genome with an identity score threshold of 97% using Bowtie 2.[25]

Microbial taxonomic profiling and quantification have been performed on the resulting reads using MetaPhlAn 3.0.[25] The samples were subsequently filtered to remove any samples containing a countable species level below 3 standard deviations from the mean.

Additionally, we compared the role of *H. pylori* seropositivity in terms of relative abundance of key immunotherapy-related bacteria:[9–11] *Bifidobacterium longum, Faecalibacterium prausnitzii, Ruminoccocus genus, Streptococcus parasanguinis and Streptococcus salivarius. Akkermansia muciniphila* (Akk) trichotomic analysis was conducted in the NSCLC cohort using the same thresholds as published by *Derosa et al. Nature Medicine 2022*.[9] All these analyses were conducted using a Mann–Whitney U test. *H. pylori* Pos and *H. pylori* Neg groups were subsequently analyzed in terms of these groups using a Chi-square test.

H. Pylori IgG virulent factors testing

We performed the *recom*Line Helicobacter IgG 2.0 line immunoassay on the serum of 20 patients with melanoma to identify specific antibodies against ten selected antigens of *Helicobacter pylori* (such as cytotoxin-associated gene A; CagA, Vacuolating cytotoxin; VacA and GroEL).[26] The evaluation of the presence of horizontal gene transfer in the stools was performed by retrieving known virulence factors listed by Chang *et al.* DNA sequences on NCBI's Gene Webservice (VacA, CagA, T4SS, BabA, and DupA).[27,28] These sequences were subsequently compared to the patient microbiome data using BLAST and Daisy.[29,30]

Statistically Analysis

Proportions and means were compared by the chi-square test, or t-test, for categorical and continuous variables, respectively. Objective response rate (ORR) was defined as the sum of complete (CR) and partial (PR) responses according to RECIST 1.1. OS was defined as time between ICI initiation and death from any cause. Progression-free survival (PFS) was defined as the time between ICI initiation and tumor progression (defined using RECIST 1.1), or death, whichever occurred first. Survival functions for OS and PFS were estimated with the Kaplan–Meier estimator. Log-rank test was used to compare survival distributions between groups. The association between clinical and biological variables and survival outcomes was assessed with univariate Cox models.

Metagenomic analyses were performed with the phyloseq R package.[31] Shannon and Inverse Simpson indexes was used as alpha-diversity measurements at the species level. The Wilcoxon signed-rank test was used to determine significant differences of these indexes among the different groups. The Bray-Curtis dissimilarity was used as a beta-diversity measurement and compared between groups using the PERMANOVA test. Linear discriminant analysis (LDA) Effect Size (LEfSe) was used to perform differential microbial abundance analysis. All statistics and figures were performed using R v4.1.0 and GraphPad Prism v.8.3.1. A p-value ≤0.05 was considered as statistically significant.

Results

Association between the *H. pylori* seropositivity status and clinical outcomes in the melanoma cohort

In this cohort of 97 patients with advanced melanoma, the median follow-up was 29.9 months and the majority of patients received ICI in the first-line setting (80%), with 81% of patients treated with anti-PD-1 monotherapy, while 12% received the combination of anti-PD-1 plus anti-CTLA-4. The mean age of the studied population was 61 years, with most patients having an ECOG performance status of 0 (81%) (Table 1).

Table 1. Baseline characteristics of patients with advanced melanoma (n = 97).

Characteristics	Total n = 97	*H. pylori* Neg n = 76	*H. pylori* Pos n = 21	*p*-Value
Age at ICI initiation, years[a]	61 (13.5)	61 (13.7)	62 (13.8)	*0.74*
Sex[b]				
Female	36 (37)	31 (41)	5 (24)	*0.15*
Male	61 (63)	45 (59)	16 (76)	
ECOG performance-statusscore[b]				
0	74 (76)	59 (78)	15 (71)	*0.55*
1	23 (24)	17 (22)	6 (29)	
Stage[b]				
IIIb	12 (12)	10 (13)	2 (10)	*0.75*
IIIc	15 (15)	11 (14)	4 (19)	
IV	70 (73)	55 (73)	15 (71)	
BRAF status[b]				
Wild-type	56 (64)	43 (63)	13 (65)	*0.89*
Mutated	32 (36)	25 (37)	7 (35)	
NA	*9*	*8*	*1*	
LDH at ICI (N < 215)[b]				
< ULN	72 (76)	55 (74)	17 (81)	*0.77*
> ULN	23 (24)	19 (26)	4 (19)	
NA	*2*	*2*	*1*	
Brain metastasis[b]				
No	81 (84)	61 (80)	20 (95)	*0.18*
Yes	16 (16)	15 (20)	1 (5)	
First line ICI[b]				
No	19 (20)	15 (20)	4 (19)	*1.00*
Yes	78 (80)	61 (80)	17 (81)	
ICI[b]				
Anti-PD-1	79 (81)	59 (78)	20 (95)	*0.42*
Anti-CTLA-4	6 (7)	6 (8)	0 (0)	
ICI combinaison	12 (12)	11 (14)	1 (5)	
Antibiotics[b]				
No	91 (94)	71 (93)	20 (95)	*1.00*
Yes	6 (6)	5 (7)	1 (5)	
Proton Pomp Inhibitors[b]				
No	83 (85)	63 (83)	20 (95)	*0.29*
Yes	14 (15)	13 (17)	1 (5)	

Notes: [a]Expressed in mean (SD). Student's t test p-value. [b]Expressed in n (%). Chi-square test *p*-value. ICI: Immune Checkpoint Inhibitor.

H. pylori antigen-directed IgG antibodies were detected in the plasma of 21 (22%) patients. Baseline patient characteristics between *H. pylori* Pos vs. *H. pylori* Neg were well balanced between both groups (Table 1). Importantly, there was no difference of antibiotic (ATB) exposure 30 days prior to ICI initiation in both groups ($p = 1.00$). In addition, 14 patients were on proton pomp inhibitors (PPI) prior to ICI initiation, and it was well balanced between both groups ($p = .29$) (Table 1).

Next, we assessed the impact of *H. pylori* serologic status on the clinical outcomes. First, ORR was numerically decreased in the *H. pylori* Pos compared to *H. pylori* Neg group, 57% and 75%, respectively ($p = .11$) (Figure 1a). Second, in *H. pylori* Pos, OS was significantly shorter compared to *H. pylori* Neg with a median OS of 44.1 months and not reached (NR), respectively (HR 3.21 95% CI 1.15–8.97, $p = .02$) (Figure 1b). There was a trend toward shorter PFS in the *H. pylori* Pos group compared to the *H. pylori* Neg group 22.2 months vs NR (HR 1.92 95% CI 0.95–3.92, $p = .07$) (Figure 1c).

Then, we addressed the potential association between irAEs and *H. pylori* seropositivity. Seventeen patients (18%) had grade 3–4 irAEs. There was no association between *H. pylori* status and the occurrence of irAEs ($p = .35$) (Figure 1d).

Next, we performed a subgroup analysis taking into consideration only the patients treated in the first-line setting (n = 78). In this sub-group of 78 patients, 17 (22%) patients were *H. pylori* Pos and baseline characteristics were well balanced between *H. pylori* groups (Supp. Table 1). ORR was numerically decreased in the *H. pylori* Pos group compared to *H. pylori* Neg group (64% vs 82%, $p = .38$) (Figure 2a). OS was significantly shorter in the *H. pylori* Pos (HR 5.45, 95% CI 1.47–20.2, $p = .005$) (Figure 2b). PFS was numerically shorter in the *H. pylori* Pos patients (22.2 months vs NR, HR 2.13 95% CI 0.87–5.21, $p = .09$) (Figure 2c). Lastly, there was no association between irAEs and *H. pylori* status ($p = .75$) (Figure 2d).

H. pylori seropositivity does not impact fecal microbiome diversity

We subsequently sought to understand the potential association between fecal microbiome composition and *H. pylori* seropositivity. Comparison of the fecal microbiome content was performed on patients with melanoma (n = 43; n = 13 patients *H. pylori* Pos compared to n = 30 patients *H. pylori* Neg). There was no difference in alpha diversity metrics between *H. pylori* Pos and *H. pylori* Neg groups (Shannon: $p = .99$ and Inverse Simpson: $p = .74$, Figure 3a). Beta diversity analysis between both groups showed no significant cluster difference following statistical analysis ($p = .20$, Figure 3b). Differential abundance analyses showed an increase of *Dorea formicigenerans, Eubacterium ventriosum*, and *Ruminococcus (Mediterraneibacter) torques* in the *H. pylori* Pos group while a significant enrichment of *Alistipes finegoldii, Clostridium sp. CAG 58, Blautia producta* and *Hungatella hathewayi* in the *H. pylori* Neg group was observed (Figure 3c). In addition, we assessed the relative abundance of known bacteria associated

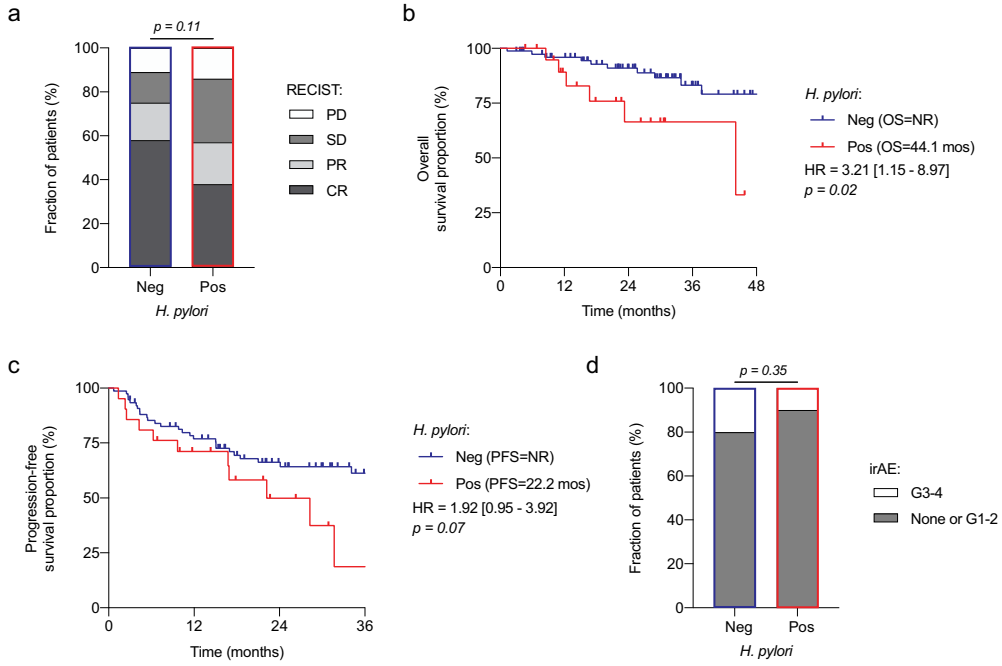

Figure 1. Association between the *H. pylori* seropositivity status and clinical outcomes in 97 patients with advanced melanoma. A. Stacked barplot between H. pylori seropositivity status in terms of ORR in 97 patients with advanced melanoma. CR; complete response. PR; partial response, SD; stable disease, PD; progressive disease analyzed using Chi-square test. **B**. Kaplan–Meier curve of overall survival in 97 patients with advanced melanoma. **C**. Kaplan–Meier curve of progression-free survival in 97 patients with advanced melanoma. **D**. Stacked barplot between H. pylori seropositivity status in terms of autoimmune toxicities in 97 patients with advanced melanoma.

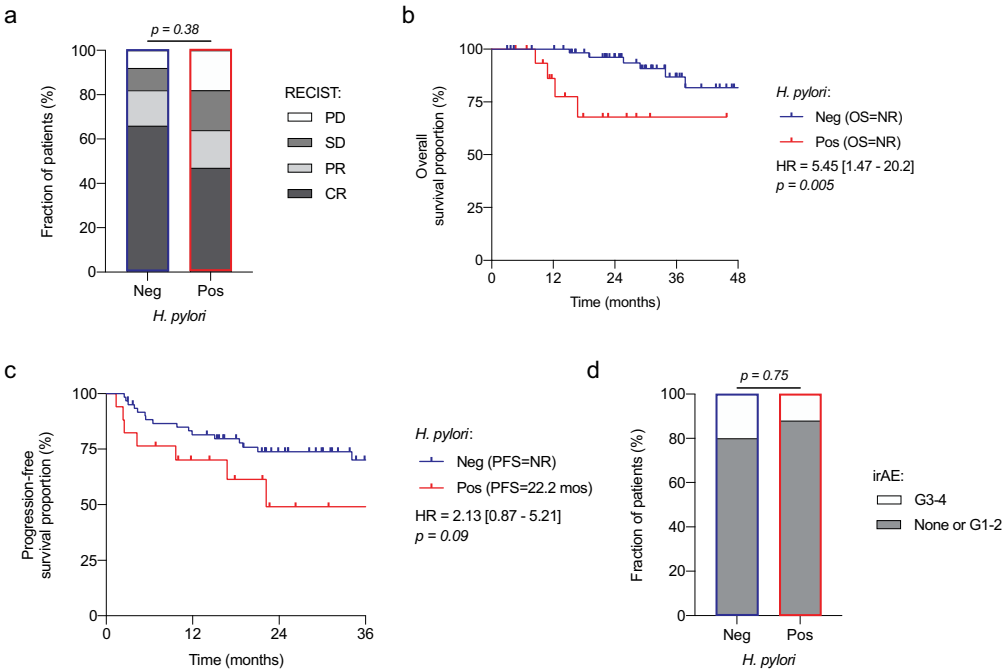

Figure 2. Association between the H. pylori seropositivity status and clinical outcomes in 78 patients with advanced melanoma treated with first-line ICI. A. Stacked barplot between H. pylori seropositivity status in terms of ORR in 78 patients with advanced melanoma treated with first-line ICI. CR; complete response. PR; partial response, SD; stable disease, PD; progressive disease analyzed using Chi-square test. **B**. Kaplan–Meier curve of overall survival in 78 patients with advanced melanoma treated with first-line ICI. **C**. Kaplan–Meier curve of progression-free survival in 78 patients with advanced melanoma treated with first-line ICI. **D**. Stacked barplot between H. pylori seropositivity status in terms of autoimmune toxicities in 78 patients with advanced melanoma treated with first-line.

with ICI response such as *Bifidobacterium longum, Faecaliacterium prausnitzii and Ruminococcus genus* between *H. pylori* Pos and *H. pylori* Neg patients and showed there was no difference ($p = .46$, *0.35* and *0.38*, respectively) (Figure 3d, 3E Supp. Fig. 1A). Then, we analyzed the content of *Streptococcus parasanguinis* and *Streptococcus salivarius* which correlated with ICI primary resistance in melanoma.[11] No difference was observed between *H. pylori* Pos and Neg groups ($p = .62$ and $p = .78$, respectively) (Supp. Fig. 1B and 1C).

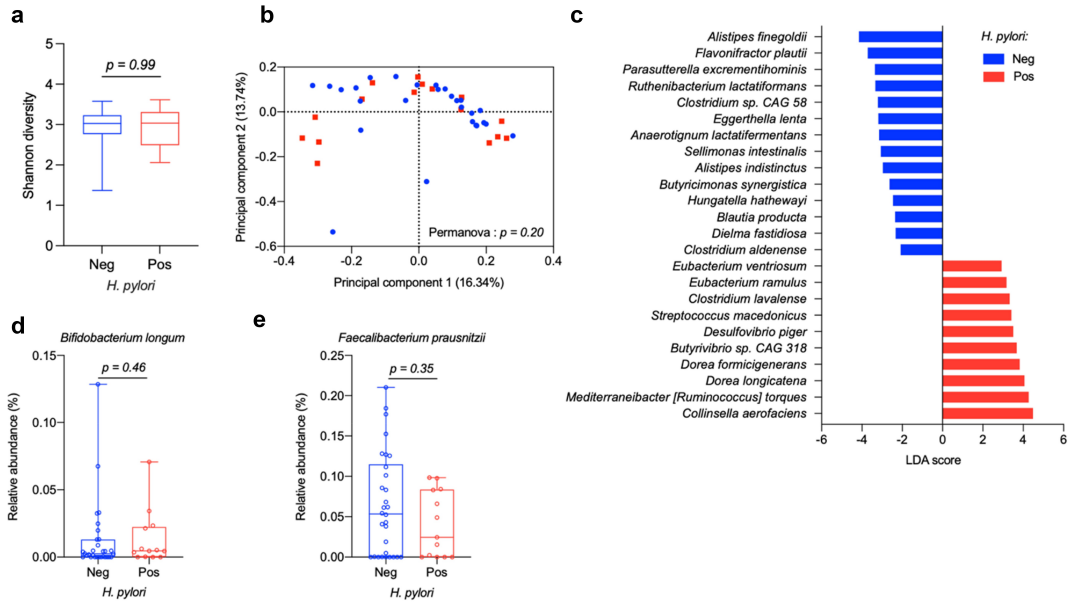

Figure 3. Gut microbiome composition for *H. pylori* Pos and *H. pylori* Neg patients in melanoma cohort. A. Alpha diversity of *H. pylori* Pos and *H. pylori* Neg patients (n = 43). The bold line represents the median. The bottom and top hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The top whisker extends from the hinge to the largest value no further than 1.5 * interquartile range from the hinge. **B**. Beta diversity for 43 patients with melanoma stratified by *H. pylori* seropositivity. **C**. Differential abundance analysis using LEfSe stratified by *H. pylori* status. Only species were displayed. Note that the presence of each bacterium on the LEfSe denotes statistical significance ($p < .05$). **D**. Relative abundance of *Bifidobacterium longum* between *H. pylori* Pos and *H. pylori* Neg patients. **E**. Relative abundance of *Faecalibacterium prausnitzii* between *H. pylori* Pos and *H. pylori* Neg patients.

To strengthen these observations, we performed the same analytical pipeline in an independent cohort using data obtained from 29 patients with NSCLC published in Oster *et al.*[18] In this cohort, eight patients were *H. pylori* Pos, and the PFS was decreased in the *H. pylori* Pos patients compared to *H. pylori* Neg group (2.1 vs 9.2 months HR = 2.39, 95% CI 1.01–5.65, $p = .05$). When we analyzed the fecal microbiome profiling of these patients according to *H. pylori* seropositivity, we did not observe any significant change in terms of alpha diversity (Shannon: $p = .94$ and Inverse Simpson: $p = .82$, Figure 4a) and absence of unique cluster for beta diversity analysis ($p = .58$, Figure 4b). The LEfSe analysis on the cohort demonstrated a significant increase in *Phascolarctobacterium sp. CAG 266, Hungatella hathewayi, Bacteroides galacturonicus*

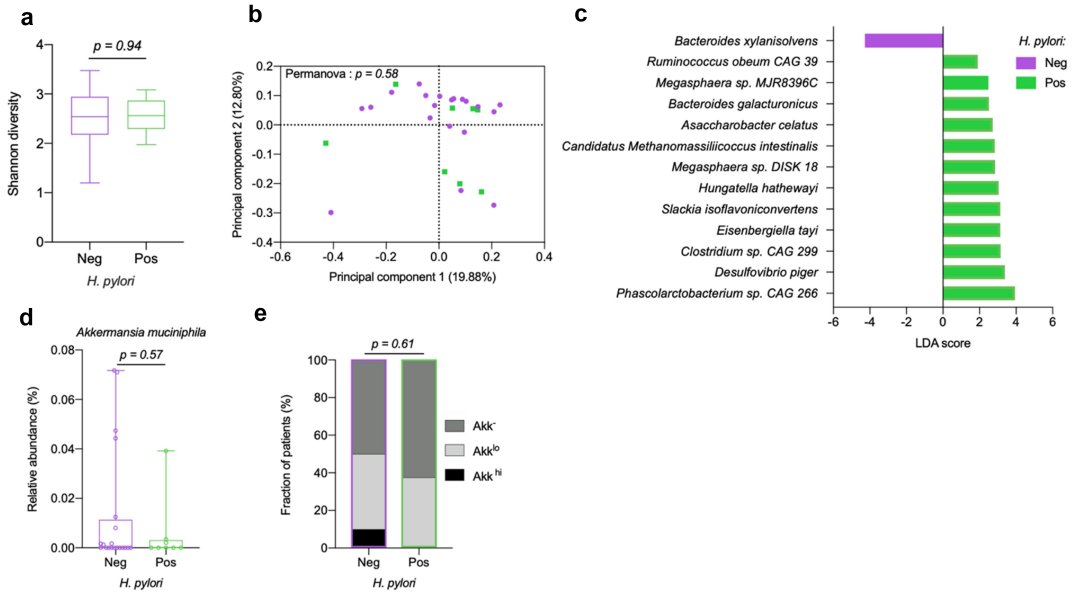

Figure 4. Gut microbiome composition for *H. pylori* Pos and *H. pylori* Neg patients in lung cohort. Alpha diversity of *H. pylori* Pos and *H. pylori* Neg NSCLC patients (n = 28). The bold line represents the median. The bottom and top hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The top whisker extends from the hinge to the largest value no further than 1.5 * interquartile range from the hinge. **B**. Beta diversity for 28 NSCLC patients stratified by *H. pylori* seropositivity. **C**. Differential abundance analysis using LEfSe stratified by *H. pylori* status. Only species were displayed. Note that the presence of each bacterium on the LEfSe denotes statistical significance ($p < .05$). **D**. Relative abundance of *Akkermansia muciniphila* between *H. pylori* Pos and *H. pylori* Neg patients. **E**. Trichotomic analysis of *Akkermansia muciniphila* between *H. pylori* Pos and *H. pylori*Neg patients.

and *Clostridium sp. CAG 299* in the *H. pylori* Pos group. Conversely, in the *H. pylori* Neg group, we observed an increase in *Bacteroides xylanisolvens* (Figure 4c). Then, we investigated the relative abundance of *Akkermansia muciniphila*, where no difference was observed ($p = .57$) (Figure 4d). Next, we represented *Akkermansia muciniphila* in a trichotomic stratification as previously published[9] and showed no correlation with *H. Pylori* status ($p = .61$) (Figure 4e).

Altogether, in the melanoma and lung cohorts, our results suggest that beneficial bacteria with known immune-potentiating effects such as *Eubacterium, Dorea, Hungatella* and *Alistipes* were increased independently of *H. pylori* status (Figure 3c, Figure 3d).[7,9–11,32,33]

Finally, we tried to detect *H. pylori* virulence factor such as VacA, CagA, T4SS on the serum and metagenomic raw data.

In 20 *H. pylori* seropositive patients, immunoassay revealed that 18 (90%) patients were tested positive for at least one *H. pylori* virulent antigen. Using the manufacturer brochure, 16 patients tested positive with 9 (56%) patients presented a type I (express virulent CagA or VaCA protein) and 7 (44%) patients presented a type II (does not express virulent CagA or VaCA protein) infection. Eight (40%) patients were positive for CagA, 4 (20%) patients were positive for VacA and 17 (85%) were positive for GroEL. Therefore, there is a possibility that four (20%) patients may have been misclassified as a false-positive with the ELISA test.

To determine if this new analysis could impact our results, we reanalyzed the data and included these four patients in the *H. pylori* Neg group. Nevertheless, OS was significantly shorter compared to *H. pylori* Neg with a median OS of 44.1 months and not reached, respectively (HR 3.38 95% CI 0.74–15.5, $p = .01$) (Supp. Fig. 2A). Of note, we did not find any difference in OS based on the type I and type II infection ($p = .59$) (Supp. Fig. 2B). However, we were unable to demonstrate that any of the *H. pylori* sequences were horizontal gene transfer in the fecal samples sequenced.

Discussion

First, the results of our study suggest that *H. pylori* seropositivity status was associated with shorter OS in patients with melanoma. Second, the worse clinical outcomes observed in *H. pylori* Pos group were independent of prior ATB exposure, an important finding given that ATB have been associated with worse response to ICI in a meta-analysis of more than 11,000 patients.[34] Third, neither alpha- nor beta-diversities were different in *H. pylori* groups providing no evidence of a unique microbiome cluster present in each group. Finally, in order to validate that this association was independent on the gut microbiome, we found similar results in one independent NSCLC cohort.

Our finding of the association of *H. pylori* Pos status with negative outcome supports prior work from two cohorts of 29 and 60 patients with NSCLC amenable to ICI whereby *H. pylori* Pos patients had decrease ICI efficacy.[18] Oster et al. further demonstrated in preclinical model that *H. pylori* infection reduced the activation of $CD8^+$T cells by altering the cross-presentation activities of DC and affected the innate and adaptive immune response of the infected mice.[18] Moreover, a recent paper showed in a cohort of 77 patients with gastric cancer treated with anti-PD -1, that *H. pylori* seropositive was associated with a shorter OS and PFS.[19] Furthermore, from recent large microbiome studies in melanoma and NSCLC patients, we observed bacteria associated with favorable response such as *Eubacterium ventriosum, Ruminococcus (Mediterraneibacter) torques, Dorea formicigenerans* significantly overrepresented in *H. pylori* Pos patients.[11,35] Moreover, *Hungatella hathewayi* was overrepresented in *H. pylori* Neg patients. This bacterium is known to be increased post-ATB exposure in NSCLC and RCC and was associated with ICI resistance.[32,33]

In the additional metagenomic we performed in the independent NSCLC cohort, we did not observe any evidence of unique bacteria cluster based on *H. pylori* status. Furthermore, *Hungatella hathewayi* was, in this cohort, increased in the *H. pylori* Pos group, however *Bacteroides xylanisolvens* was increased in *H. pylori* Neg group. *Bacteroides xylanisolvens* has recently been associated with resistance in a large cohort of 94 patients with melanoma.[10] The absence of unique microbiome signature according to *H. pylori* status was previously demonstrated in murine experiments.[18] Murine infections of *H. pylori* were less responsive to either anti-CTLA4/PD-L1 combination therapy or Ova specific $CD8^+$ T cells (OT-1 cells) vaccine. Two strategies to show an absence of the *H. pylori*-mediated immunosuppression relied on the microbiome were used. Co-housing of infected and not infected mice did not alter the immunosuppressive phenotype of *H. Pylori* (co-housing did not induce *H. pylori* transmission). Next, 16S rRNA did not show any influence in microbiome composition in *H. pylori* infected animal.[18] Taken together, these findings are in-line with our findings that *H. pylori* associated with OS in our cohort, and that this association was independent on the gut microbiome.

We observed a discordance between *H. pylori* seropositivity on ELISA test and on immunoassay. Quantitative IgG detection represents the standard of care in routine clinical setting while immunoassay specific for *H. pylori* remains a research tool. Only, two patients had no specific IgG response to any virulent factors and the discrepancy in the test is most likely secondary to sensitivity threshold used in the routine ELISA testing or a lost of IgG response to specific virulent factor in an elderly patient population. Nevertheless, the correlation with OS and *H. pylori* seropositive patients remained significant using both kits.

Questions also remain regarding the impact of different *H. pylori* strains and geodistribution of the population. Indeed, the *H. pylori* immune potentiating effect has been associated with refractory immune thrombocytopenia purpura (ITP).[36] Even if society guidelines recommend eradication of *H. pylori* in this setting, several observational studies from different countries demonstrated that *H. pylori* eradication did not have the same beneficial effect on ITP leading to the hypothesis that different *H. pylori* strains may have differing immune effects.[37]

Despite determining the impact of *H. pylori* on a separate cohort of patients treated with ICI, this study contains several limitations. First, we only tested serum *H. pylori* and did not distinguish between eradicated vs chronic infection, and our conclusions are only applicable to IgG seropositivity. Second, we only had access to fecal microbiome, and therefore our analysis did not take the upper GI tract microbiome into account. Last, we did not take into account factors which may influence seropositivity, such as socioeconomic factors, diet or other risk factors for *H. pylori*.

Altogether, our study is the first to report the negative impact of *H. pylori* on OS in patients with melanoma amenable to ICI and to confirm similar findings in a separate cancer histology. Furthermore, we demonstrated that *H. pylori* did not seem to have any potent impact on the fecal microbiome and more mechanistic experiments are needed. The data presented in our study only provide hypothesis-generating data and further need to perform additional trials to confirm this observation. Our findings warrant validation in large prospective observational cohort in patients with ICI, comparing the outcomes for melanoma patients between *H. pylori* Pos and *H. pylori* Neg patients and reinforce the need to develop novel strategies to eradicate *H. pylori* in cancer patients.[38]

Data Availability Statement

Data are available under the BioProject PRJNA800058. https://www.ncbi.nlm.nih.gov/bioproject/800058

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References

1. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, Lao CD, Schadendorf D, Wagstaff J, Dummer R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19(11):1480–1492. doi:10.1016/S1470-2045(18)30700-9.
2. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–330. doi:10.1056/NEJMoa1412082.
3. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2019;381(16):1535–1546. doi:10.1056/NEJMoa1910836.
4. Morad G, Helmink BA, Sharma P, Wargo JA. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. Cell. 2021;184(21):5309–5337. doi:10.1016/j.cell.2021.09.020.
5. Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov. 2022;12(1):31–46. doi:10.1158/2159-8290.CD-21-1059.
6. Derosa L, Routy B, Kroemer G, Zitvogel L. The intestinal microbiota determines the clinical efficacy of immune checkpoint blockers targeting PD-1/PD-L1. OncoImmunology. 2018;7(6):e1434468. doi:10.1080/2162402X.2018.1434468.
7. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science. 2018;359(6371):91–97. doi:10.1126/science.aan3706.
8. Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen WS, Derosa L, Khan MAW, Cogdill AP, White MG, Wong MC, et al. Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. Nat Med. 2021;27(8):1432–1441. doi:10.1038/s41591-021-01406-6.
9. Derosa L, Routy B, Thomas AM, Iebba V, Zalcman G, Friard S, Mazieres J, Audigier-Valette C, Moro-Sibilot D, Goldwasser F, et al. Intestinal Akkermansia muciniphila predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. Nat Med. 2022;28(2):315–324. doi:10.1038/s41591-021-01655-5.
10. McCulloch JA, Davar D, Rodrigues RR, Badger JH, Fang JR, and Cole AM, et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. Nat Med. 2022:28:1–12.
11. Lee KA, Thomas AM, Bolte LA, Björk JR, de Ruijter LK, Armanini F, Asnicar F, Blanco-Miguez A, Board R, and Calbet-Llopart N, et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. Nat Med. 2022:28;1–10.
12. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, Adler K, Dick-Necula D, Raskin S, Bloch N, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2021;371(6529):602–609. doi:10.1126/science.abb5920.
13. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, Deblasio RN, Menna C, Ding Q, Pagliano O, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science. 2021;371(6529):595–602. doi:10.1126/science.abf3363.
14. Messaoudene M, Pidgeon R, Richard C, Ponce M, Diop K, Benlaifaoui M, Nolin-Lapalme A, Cauchois F, Malo J, and Belkaid W, et al. A natural polyphenol exerts antitumor activity and circumvents anti–PD-1 resistance through effects on the gut microbiota. Cancer Discov. 2022;14:OF1–18.
15. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983;1:1273–1275.
16. Guevara B, Cogdill AG. *Helicobacter pylori*: a review of current diagnostic and management strategies. Dig Dis Sci. 2020;65(7):1917–1931. doi:10.1007/s10620-020-06193-7.
17. Moyat M, Velin D. Immune responses to *Helicobacter pylori* infection. World J Gastroenterol. 2014;20(19):5583–5593. doi:10.3748/wjg.v20.i19.5583.
18. Oster P, Vaillant L, Riva E, McMillan B, Begka C, Truntzer C, Richard C, Leblond M, Messaoudene M, and Machremi E, et al. *Helicobacter pylori* infection has a detrimental impact on the efficacy of cancer immunotherapies. Gut. 2021;12:gutjnl-2020–323392.
19. Association of *Helicobacter pylori* infection with survival outcomes in advanced gastric cancer patients treated with immune checkpoint inhibitors [Internet]. 2022 [cited 2022 May 21]. Available from: https://www.researchsquare.com
20. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–247. doi:10.1016/j.ejca.2008.10.026.
21. Common Terminology Criteria for Adverse Events (CTCAE). 2017:147.

22. Costea PI, ZellerG, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen M, Hercog R, Jung F-E, et al. Towards standards for human fecal sample processing in metagenomic studies. Nat Biotechnol. 2017 Nov;35(11):1069–1076. doi:10.1038/nbt.3960.
23. Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Dore J. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol. 1999;65(11):4799–4807. doi:10.1128/AEM.65.11.4799-4807.1999.
24. Criscuolo A, Brisse S. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. Genomics. 2013;102(5–6):500–506. doi:10.1016/j.ygeno.2013.07.011.
25. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357–359. doi:10.1038/nmeth.1923.
26. Formichella L, Romberg L, Meyer H, Bolz C, Vieth M, Geppert M, Göttner G, Nölting C, Schepp W, Schneider A, et al. Validation of a novel immunoline assay for patient stratification according to virulence of the infecting *helicobacter pylori* strain and eradication status. J Immunol Res. 2017;2017:8394593. doi:10.1155/2017/8394593.
27. Chang WL, Yeh YC, Sheu BS. The impacts of H. pylori virulence factors on the development of gastroduodenal diseases. J Biomed Sci. 2018;25(1):68. doi:10.1186/s12929-018-0466-9.
28. Brown GR, Hem V, Katz KS, Ovetsky M, Wallin C, Ermolaeva O, Tolstoy O, Tatusova T, Pruitt KD, and Maglott DR, et al. Gene: a gene-centered information resource at NCBI. Nucleic Acids Res. 2015;43(1):D36–42. doi:10.1093/nar/gku1055.
29. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–410. doi:10.1016/S0022-2836(05)80360-2.
30. Trappe K, Marschall T, Renard BY. Detecting horizontal gene transfer by mapping sequencing reads across species boundaries. Bioinformatics. 2016;32(17):i595–604. doi:10.1093/bioinformatics/btw423.
31. McMurdie PJ, Holmes S, Watson M. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217. doi:10.1371/journal.pone.0061217.
32. Derosa L, Routy B, Fidelle M, Iebba V, Alla L, Pasolli E, Segata N, Desnoyer A, Pietrantonio F, and Ferrere G, et al. Gut bacteria composition drives primary resistance to cancer immunotherapy in renal cell carcinoma patients. Eur Urol. 2020;78(2):195–206. doi:10.1016/j.eururo.2020.04.044.
33. Hakozaki T, Richard C, Elkrief A, Hosomi Y, Benlaïfaoui M, Mimpen I, Terrisse S, Derosa L, Zitvogel L, and Routy B, et al. The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer. Cancer Immunol Res. 2020;8(10):1243–1250. doi:10.1158/2326-6066.CIR-20-0196.
34. Derosa L, Routy B, Desilets A, Daillère R, Terrisse S, and Kroemer Get al, Microbiota-Centered Interventions: The Next Breakthrough in Immuno-Oncology? Cancer Discov. 2021 Oct;11(10):2396–412.
35. Spencer CN, McQuade JL, Gopalakrishnan V, McCulloch JA, Vetizou M, and Cogdill APet al, Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. Science. 2021 Dec 24;374(6575):1632–40.
36. Stasi R, Provan D. *Helicobacter pylori* and Chronic ITP. Hematology Am Soc Hematol Educ Program. 2008;2008(1):206–211. doi:10.1182/asheducation-2008.1.206.
37. Kim BJ, Kim HS, Jang HJ, Kim JH. *Helicobacter pylori* eradication in idiopathic thrombocytopenic purpura: a meta-analysis of randomized trials. Gastroenterol Res Pract. 2018;2018:6090878. doi:10.1155/2018/6090878.
38. Kosunen TU. Antibody titres in *Helicobacter pylori* infection: implications in the follow-up of antimicrobial therapy. Ann Med. 1995;27(5):605–607. doi:10.3109/07853899509002477.