Research article

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Association between antibiotics and adverse oncological outcomes in patients receiving targeted or immune-based therapy for hepatocellular carcinoma

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Background & Aims: Immune checkpoint inhibitors (ICIs) alone or in combination with other ICIs or vascular endothelial growth factor pathway inhibitors are therapeutic options in unresectable/metastatic hepatocellular carcinoma (HCC). Whether antibiotic (ATB) exposure affects outcome remains unclear.
Methods: This study retrospectively analysed an FDA database including 4,098 patients receiving ICI (n = 842) either as monotherapy (n = 258) or in combination (n = 584), tyrosine kinase inhibitor (TKI) (n = 1,968), vascular endothelial growth factor pathway inhibitors (n = 480), or placebo (n = 808) as part of nine international clinical trials. Exposure to ATB within 30 days before or after treatment initiation was correlated with overall survival (OS) and progression-free survival (PFS) across therapeutic modality before and after inverse probability of treatment weighting (IPTW).
Results: Of 4,098 patients with unresectable/metastatic HCC, of which 39% were of hepatitis B aetiology and 21% were of hepatitis C aetiology, 83% were males with a median age of 64 years (range 18–88), a European Collaborative Oncology Group performance status of 0 (60%), and Child–Pugh A class (98%). Overall, ATB exposure (n = 620, 15%) was associated with shorter median PFS (3.6 months in ATB-exposed *vs.* 4.2 months; hazard ratio [HR] 1.29; 95% CI 1.22, 1.36) and OS (8.7 months in ATB-exposed *vs.* 10.6 months; HR 1.36; 95% CI 1.29, 1.43). In IPTW analyses, ATB was associated with shorter PFS in patients treated with ICI (HR 1.52; 95% CI 1.34, 1.73), TKI (HR 1.29; 95% CI 1.19, 1.39), and placebo (HR 1.23; 95% CI 1.11, 1.37). Similar results were observed in IPTW analyses of OS in patients treated with ICI (HR 1.22; 95% CI 1.08, 1.38), TKI (HR 1.40; 95% CI 1.30, 1.52), and placebo (HR 1.40; 95% CI 1.25, 1.57).
Conclusions: Unlike other malignancies where the detrimental effect of ATB may be more prominent in ICI recipients, ATB is associated with worse outcomes in this study across different therapies for HCC including placebo. Whether ATB is causally linked to worse outcomes through disruption of the gut–liver axis remains to be demonstrated in translational studies.
Impact and Implications: A growing body of evidence suggests the host microbiome, frequently altered by antibiotic treatment, as an important outcome predictor in the context of immune checkpoint inhibitor therapy. In this study, we analysed the effects of early antibiotic exposure on outcomes in almost 4,100 patients with hepatocellular carcinoma treated within nine multicentre clinical trials. Interestingly, early exposure to antibiotic treatment was associated with worse outcomes not only in patients treated with immune checkpoint inhibitors but also in those treated with tyrosine kinase inhibitors and placebo. This is in contrast to data published in other malignancies, where the detrimental effect of antibiotic treatment may be more prominent in immune checkpoint inhibitor recipients, highlighting the uniqueness of hepatocellular carcinoma given the complex interplay between cirrhosis, cancer, risk of infection, and the pleiotropic effect of molecular therapies for this disease.

Keywords: Antibiotics; Cancer immunotherapy; Targeted therapy; Immune checkpoint inhibitors; Hepatocellular carcinoma; Gut microbiota; Microbial dysbiosis.
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Introduction

The advent of effective systemic therapy options has reshaped the therapeutic landscape of advanced/unresectable hepatocellular carcinoma (HCC).[1] Sequential use of tyrosine kinase inhibitor (TKI) therapy represented a breakthrough in an oncological diagnosis that had for a long time not benefited from the use of systemic therapy.[2,3] More recently, clinical testing of monoclonal antibodies in the immune checkpoint inhibitor (ICI) class targeting the programmed cell death-1 (PD-1) receptor/ligand (PD-L1) and the cytotoxic T-cell lymphocyte associated antigen-4 (CTLA-4), two key drivers of anticancer immunity,[4] has led to the understanding that a proportion of patients with HCC may respond to T-cell immune reconstitution.[5] However, in the absence of predictive biomarkers capable of identifying patients who benefit from ICIs, demonstrating the clinical utility of monotherapy regimens has been particularly difficult, especially in view of late-stage failures in clinical development.[6,7]

Clinical testing of combinations of PD-1 pathway inhibitors with blockers of the vascular endothelial growth factor (VEGF) pathway, the CTLA-4 pathway, or TKIs have risen to prominence as therapies with the potential to generate radiologically measurable responses in over one-fourth of patients with advanced HCC.[8] These combinations have also demonstrated survival benefit compared with sorafenib as illustrated in the IMbrave150 trial, which evaluated the combination of atezolizumab plus bevacizumab.[9]

As drug development in HCC continues at a rapid pace, considerable interest has been devoted to the investigation of mechanisms of response and resistance to ICI, in an effort to guide personalised therapy.[10] The specific immune-mediated mechanism of action of ICIs has led to the study of the host immune status as a source of prognostic and predictive traits that may aid clinicians in formulating therapeutic decisions in the clinic. In recipients of ICI therapy, characteristics such as pretreatment BMI,[11] the presence of a systemic pro-inflammatory status,[12,13] the emergence of treatment-related adverse events[14] are independently associated with outcomes from immunotherapy.

In addition to host factors, concomitant therapies may have a direct effect on the host immune function and have been studied for their ability to modify the efficacy of ICI or make patients more prone to developing toxicity from ICI therapy.[15,16]

Antibiotics (ATB) are among the class of drugs that exert immunomodulatory effects, for example, through perturbation of the gut microbiota.[17] Use of broad-spectrum ATB can reduce gut bacterial diversity and foster the expansion of *taxa* that may negatively affect response to ICI.[18,19] Data suggest that ATB exposure either before or early during the course of ICI therapy are associated with worse outcomes in recipients of ICI therapy in terms of objective response rate, progression-free survival (PFS), and overall survival (OS).[20–24] In indications other than HCC, it has been reported that the detrimental effect of ATB is most apparent in patients who receive ICI therapy as compared with those who receive cytotoxic chemotherapy[25] or chemo-immunotherapy combinations,[26] suggesting that ATB might exert a preconditioning effect on cancer-specific immune control.[27]

In patients with HCC the role of ATB exposure in influencing outcome from ICI is unclear.

Two previously published multicentre retrospective studies have reported conflicting results.[28,29] However, significant heterogeneity exists among published studies, where the majority of patients were treated with PD-1 monotherapy across different treatment lines and with varying degrees of liver dysfunction, a factor that affects the prognosis of ICI recipients.[30,31]

To further evaluate the strength and direction of the relationship between ATB exposure and outcome from immunotherapy we performed a patient-level analysis of international clinical trials of systemic therapy (ICI and TKI) in advanced/unresectable HCC.

Patients and methods

Study population

An internal US FDA database was used to identify clinical trials submitted to the FDA between 2016 and 2019 to support marketing applications of systemic anticancer therapies for the treatment of patients with unresectable/metastatic HCC. Nine multicentre trials were included and consisted of treatment arms including placebo, TKI, VEGF inhibitors, and PD-1/PD-L1 inhibitors as monotherapy and in combination with CTLA-4 antagonists, VEGF pathway inhibitors, or TKI. The immunotherapy agents studied included anti-PD-1, anti-PD-L1, and anti-CTLA-4 agents. The TKIs and VEGF inhibitors studied were regorafenib, lenvatinib, sorafenib, cabozantinib, ramucirumab, and bevacizumab. The different trials studied patients in the first-line setting and beyond.

Endpoint definition

OS was defined as the time from randomisation (or date of enrolment for patients in single-arm studies) to death or last recorded follow-up. PFS was defined as the time from randomisation (or enrolment for single-arm studies) to progression or death. PFS was determined according to Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 guidelines. For ATB exposure, concomitant medication records were screened to identify administration of any systemic ATB uses up to 30 days before treatment initiation. Patients who had ATB exposure, defined as exposure within 30 days before and 30 days after the initiation of any anticancer treatment, were of interest. Duration of exposure was recorded and categorised into more *vs.* less than 10 days. All clinical study-related procedures and data collection were indicated by the sponsors to have been conducted in accordance with the Declaration of Helsinki and in accordance with Good Clinical Practice.

Analysis methods

Demographic data were summarised using descriptive statistics. Categorical variables were summarised as proportions, and continuous variables were summarised using medians and ranges. For the analyses on OS and PFS, the Kaplan–Meier product limit method and log-rank tests were used to compare patients who had ATB exposure with patients who did not. Cox proportional hazard models were used to estimate the

hazard ratio (HR) of patients who had ATB exposure compared with patients who did not. All PFS and OS analyses were conducted in all patients as well as by treatment type. Unless otherwise specified, the immunotherapy group included patients receiving PD-1/PD-L1 inhibitors as monotherapy and in combination with CTLA-4 antagonists, VEGF pathway inhibitors, or TKI. Because of the inconsistent association between ATB exposure and outcome in ICI recipients in our dataset and evidence in the literature suggesting that ICI combination therapies might be less susceptible to the detrimental effects of ATB, we also evaluated ICI combination regimens separately from ICI monotherapy.

Inverse probability of treatment weighting (IPTW) was used in attempt to achieve a balanced distribution of confounders across exposure groups. The weights were derived from a propensity score IPTW model that included the following baseline variables as covariates: age, race, sex, region, European Collaborative Oncology Group (ECOG) performance status, aetiology of chronic liver disease, presence of macrovascular invasion, presence of extrahepatic disease, and receipt of prior lines of treatment. The mean squared differences (MSDs) were calculated to measure the balance in observed baseline characteristics between groups of ATB exposure. The MSDs before and after IPTW were calculated for each covariate by ATB exposure group; both unadjusted and adjusted (by IPTW) PFS and OS analyses were conducted. To validate our findings, all statistical analyses were repeated after propensity score matching considering the same potential confounders as for IPTW. All statistical analyses were conducted using the SAS software (SAS Institute Inc., Cary, NC, USA).

Results

Patients

Overall, 4,098 patients were pooled from nine selected clinical trials; among them, 1,968 (48%) received TKIs, 842 (21%) received ICI-based regimens, 480 (12%) received anti-VEGF monoclonal antibodies, and 808 (20%) received placebo. When considering antibiotic therapy exposure in the whole population, 620 patients (15%) had received ATB within the time frame of 30 days before and after first anticancer treatment or placebo was initiated, whereas the remaining 3,478 trial patients (85%) had not been exposed to ATB at all or had received them outside the time frame of interest. Rates of ATB exposure were similar across treatment types ranging from 12% of placebo to 16% of TKI and immunotherapy recipients. Duration of ATB treatment was less than or equal to 10 days in 281 of 620 exposed patients (45%) and comparable across types (Table S1).

Table 1. Baseline characteristics of clinical trial participants stratified according to antibiotic use.

	No antibiotic use n = 3,478		Antibiotic use n = 620	
Variables	**Median/n**	**Range/%**	**Median/n**	**Range/%**
Age	64	18–88	63	24–88
Race				
Missing	57	1.6	10	1.6
Asian	1,732	49.8	316	51.0
Black	59	1.7	14	2.3
White	1,432	41.2	239	38.5
Not reported	166	4.8	30	4.8
Other	32	0.9	11	1.8
Sex				
Female	538	15.5	139	22.4
Male	2,940	84.5	481	77.6
Region				
Missing	1	0.0	0	0.0
Asia	1,593	45.8	286	46.1
ROW	1,884	54.2	334	53.9
ECOG performance status				
0	2,109	60.6	347	56.0
1+	1,369	39.4	273	44.0
Child–Pugh score				
Missing	4	0.1	3	0.5
A	3435	98.8	601	96.9
B	39	1.1	16	2.6
Aetiology of chronic liver disease				
Missing	203	5.8	36	5.8
Alcohol	205	5.9	36	5.8
HBV	1,383	39.8	235	37.9
HCV	723	20.8	148	23.9
Mixed	166	4.8	29	4.7
Other	700	20.1	119	19.2
Unknown	98	2.8	17	2.7
Prior systemic therapy				
Yes	2,158	62.0	385	62.1
No	1,320	38.0	235	37.9
Macrovascular invasion				
Missing	2	0.1	1	0.2
Absence	2,495	71.7	431	69.5
Presence	981	28.2	188	30.3
Extrahepatic spread				
Absence	1,127	32.4	178	28.7
Presence	2,351	67.6	442	71.3
Type of treatment				
ICI monotherapy	225	6.5	33	5.3
ICI combination	482	13.9	102	16.5
TKI monotherapy	1,656	47.6	312	50.3
VEGF inhibitors	407	11.7	73	11.8
Placebo	708	20.4	100	16.1

ECOG, European Collaborative Oncology Group; ICI, immune checkpoint inhibitor; ROW, rest of the world; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

Baseline characteristics

Baseline characteristics of the whole population are summarised in Table 1 following stratification by ATB exposure. In the overall population, 83% (n = 3,421) of patients were male, 46% (n = 1,879) were enrolled in Asia, and 54% (n = 2,218) were from the rest of the world. Leading aetiologic factors for chronic liver disease included 39% (n = 1,618) hepatitis B infection, 21% (n = 871) hepatitis C infection, 5% (n = 195) mixed infection, 6% (n = 241) alcohol use, 20% (n = 819) other causes, 2.8% (n = 115) unknown aetiologic factors, and 6% (n = 239) missing.

At baseline, Child–Pugh class was A in 4,036 patients (98%), and most trial participants carried a diagnosis of HCC with evidence of extrahepatic spread (n = 2,793, 68%), whereas macrovascular invasion was present in 1,169 patients (29%). At baseline, 2,543 patients (62%) had received at least one line of systemic anticancer therapy.

ATB exposure is associated with worse outcome in patients who receive systemic therapy in HCC

In total, 4,036 patients (98%) were eligible for analysis of ATB exposure in relation to OS and PFS outcomes, after exclusion of 62 patients with Child–Pugh class B (n = 55) and seven patients with missing Child–Pugh class information. Patients with Child–Pugh class B disease were not examined owing to their small sample size. In all treatment modalities (ICIs, TKIs, and placebo),

Table 2. The relationship between antibiotic use and efficacy outcomes in patients with unresectable/advanced HCC.

	Unadjusted analyses		Adjusted using IPTW*	
	No antibiotic use	Antibiotic use	No antibiotic use	Antibiotic use
All patients				
Sample size				
No. of patients	3,435	601	3,171	555
Effect size	3,435	601	3,725.77	3,730.41
PFS				
Median (95% CI), months	4.2 (4.1, 4.5)	3.6 (3.0, 4.0)	4.2 (4.0, 4.4)	3.6 (2.7, 4.0)
HR† (95% CI)	1.36 (1.23, 1.50)		1.29 (1.22,1.36)	
OS				
Median (95% CI), months	10.8 (10.4, 11.3)	8.6 (7.9, 9.1)	10.6 (10.3, 11.1)	8.7 (7.8, 9.6)
HR† (95% CI)	1.42 (1.29, 1.57)		1.36 (1.29,1.43)	
Tyrosine kinase inhibitor group				
Sample size				
No. of patients	1,640	303	1,469	278
Effect size	1,640	303	1,746.74	1,752.47
PFS				
Median (95% CI), months	5.6 (5.5, 5.7)	3.7 (3.6, 4.3)	5.5 (5.4,5.6)	3.9 (3.6,4.9)
HR (95% CI)	1.40 (1.22, 1.61)		1.29 (1.19,1.39)	
OS				
Median (95% CI), months	12.2 (11.6, 13.1)	8.4 (7.1, 9.8)	11.9 (11.1,12.5)	8.8 (7.4,10.4)
HR (95% CI)	1.51 (1.31, 1.73)		1.40 (1.30,1.52)	
Immunotherapy group				
Sample size				
No. of patients	696	129	660	120
Effect size	696	129	779.56	788.67
PFS				
Median (95% CI), months	8.2 (7.6, 8.4)	8.3 (6.8, 8.9)	8.3 (7.6, 8.5)	6.8 (5.4, 8.3)
HR† (95% CI)	1.24 (0.97, 1.59)		1.52 (1.34, 1.73)	
OS				
Median (95% CI), months	11.4 (10.6, 12.1)	10.7 (9.1, 11.8)	11.5 (10.7,12.3)	10.7 (9.0,12.1)
HR† (95% CI)	1.24 (0.98,1.56)		1.22 (1.08,1.38)	
Placebo group				
Sample size				
No. of patients	697	97	675	92
Effect size	697	97	766.69	772.45
PFS				
Median (95% CI), months	1.87 (1.81, 1.93)	1.61 (1.41, 1.84)	1.9 (1.8,1.9)	1.6 (1.4,1.9)
HR (95% CI)	1.29 (1.03, 1.62)		1.23 (1.11,1.37)	
OS				
Median (95% CI), months	8.2 (7.4, 8.9)	4.4 (3.7, 6.6)	8.1 (7.3,8.9)	5.2 (3.7,7.7)
HR (95% CI)	1.58 (1.25, 1.99)		1.40 (1.25,1.57)	

ECOG, European Collaborative Oncology Group; HR, hazard ratio; IPTW, inverse probability of treatment weighting; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor.

* The weights were derived from a propensity score (IPTW) model that included the following baseline variables as covariates: age, race, sex, region, ECOG performance status, aetiology of chronic liver disease, presence of macrovascular invasion, presence of extrahepatic disease, and receipt of prior lines of treatment.

† HR is stratified by treatment group (tyrosine kinase inhibitor or immune checkpoint inhibitor alone or immune checkpoint inhibitor combination or placebo or VEGF for all patients, or immune checkpoint inhibitor alone or immune checkpoint inhibitor combination for immune checkpoint inhibitor group).

unadjusted and adjusted (by IPTW) PFS and OS analyses were conducted to compare patients by ATB exposure groups.

Baseline characteristics in the various groups were found to be generally well balanced between the ATB-exposed and ATB-unexposed groups before and after weighting (Tables S2–S4). Exceptions are among TKI recipients where ATB was more frequently administered to female patients than to male patients (21 *vs.* 15%), and to patients who received prior systemic therapy for HCC (58 *vs.* 51%), and were less frequently administered to patients who had extrahepatic disease (65 *vs.* 71%). All variables were well balanced after weighting (Table S2).

Before weighting, in the overall patient population, ATB-exposed patients (n = 601) experienced worse PFS (median, 3.6 months in ATB-exposed patients *vs.* 4.2 months; HR 1.36; 95% CI 1.23, 1.50) and worse OS (median, 8.6 months in ATB-exposed patients *vs.* 10.8 months; HR 1.42; 95% CI 1.29, 1.57) than did their ATB-unexposed counterparts (n = 3,435). After IPTW, the survival estimates in ATB-exposed patients (n = 555) *vs.* ATB-unexposed patients (n = 3,171) were comparable with the unadjusted results for both PFS (median, 3.6 months in ATB-exposed patients *vs.* 4.2 months; HR 1.29; 95% CI 1.22, 1.36) and OS (median, 8.7 *vs.* 10.6 months; HR 1.36; 95% CI 1.23, 1.43).

ATB exposure is associated with worse efficacy and survival across various therapeutic modalities

To determine whether the effect of ATB exposure on PFS and OS seen in the overall patient population could be separately

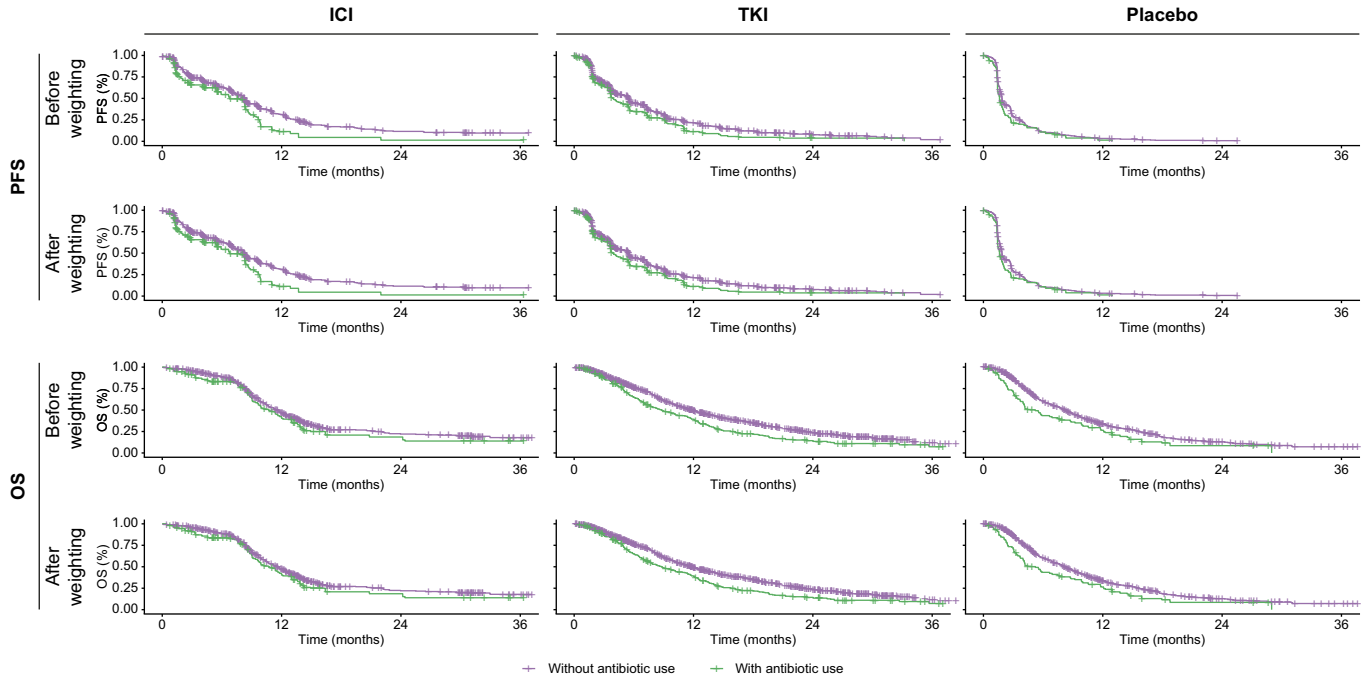

Fig. 1. Kaplan–Meier curves illustrating estimates for PFS and OS stratified according to antibiotic exposure before and after propensity score weighting in patients who received ICIs, TKIs, and placebo. ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

reproduced in patients treated with ICI, TKI, and placebo, we performed unmatched estimates for PFS in ATB-exposed *vs.* unexposed patients.

As shown in Table 2, worse PFS and OS in patients treated with TKIs and placebo was observed, whereas for the ICI group, median PFS (median, 8.3 months in ATB-exposed patients *vs.* 8.2 months; HR 1.24; 95% CI 0.97, 1.59) and OS (median, 10.7 months in ATB-exposed patients *vs.* 11.4 months; HR 1.24; 95% CI 0.98, 1.56) was not significantly different.

After adjusting for baseline differences using IPTW, estimates for PFS and OS showed a consistent worsening in ATB-exposed patients *vs.* ATB-unexposed counterparts. Fig. 1 illustrates Kaplan–Meier estimates of PFS and OS by ATB exposure groups across TKI-treated, ICI-treated, and placebo-treated cohorts.

Next, we evaluated ICI combination regimens separately from ICI monotherapy.

As shown in Table S5, the unadjusted analyses indicate an association between ATB exposure and worse PFS from ICI monotherapy regimens (median, 2.1 months in ATB-exposed patients *vs.* 4.0 months; HR 1.51; 95% CI 1.00, 2.28). Following IPTW, we found evidence of worse PFS and OS outcomes in ICI monotherapy and combination therapy for patients with ATB exposure (Fig. S1).

Comparable results were also observed after propensity score matching (Tables S6–S10).

Discussion

The liver immune microenvironment is naturally geared towards spontaneous immune suppression. As chronic liver disease worsens over time, increased intestinal permeability, bacterial overgrowth, or impaired clearance of microbial metabolites by Kupffer cells may increase the translocation of gut microbial species, leading to unopposed pro-inflammatory signalling within the liver and systemically.[32,33] An altered intestinal homoeostasis not only is pathogenic in HCC but is intimately linked to the natural progression and prognosis of liver cancer.[34] Although evidence gathered in other tumours has revealed an intimate relationship between diversity and taxonomic features of the gut microbiome and responsiveness to ICI, data on HCC are still preliminary.[35]

Similarly, the effect of ATB, a broad class of therapies associated with the highest disruption of and potential to induce long-lasting changes to the gut microbiome,[36] has not been univocally confirmed to affect responsiveness and survival of patients with HCC treated with ICI.[28,29]

In this study, the first to evaluate the relationship between ATB exposure and outcomes in a large series of patients prospectively recruited to landmark clinical trials of unresectable/advanced HCC, we provide data that ATB exposure within 30 days of treatment initiation is associated with worse PFS and OS across patients treated with a wide variety of systemic therapies for HCC including TKI and ICI monotherapy and combinations.

In ICI recipients, the negative association between ATB and survival outcomes was preserved across ICI monotherapy and combinations including dual PD-1/VEGF pathway blockade, the novel standard of care in advanced HCC.[10]

In patients receiving immunotherapy, enrichment of certain stool bacterial species including *Akkermansia*, *Ruminococcus*, and *Bifidobacteria* is associated with a higher likelihood of response from ICI.[19,37,38] The selective pressure of broad-spectrum ATB exposure on the gut microbiome leads to the expansion of *Bacteroides* and commensal *Clostridia*,[39] which can in turn facilitate recruitment and activation of immunosuppressive cells including T-regulatory and myeloid-derived suppressor cells – all negatively associated with ICI response.[40] One recent study suggested faecal microbial transplantation to restore sensitivity to PD-1

inhibition in patients with advanced melanoma,[41] potentially highlighting the central role of the gut microbiome in driving adaptive resistance to ICI.

Although the relationship between ATB and outcomes is supported by mechanistic studies and analyses of clinical data in ICI recipients, the finding of a detrimental effect across multiple treatment arms including placebo in our study is in contrast with evolving evidence in oncological indications other than HCC, where the detrimental effect of ATB pretreatment on response and survival is restricted to ICI therapy and not to chemotherapy[25] or targeted therapies unless prescribed after immune-modulating therapies.[42] Previously published retrospective evidence in sorafenib recipients had suggested that receipt of broad-spectrum ATB therapy before sorafenib was associated with worse survival in patients with advanced HCC,[43] concordantly with our study data.

Reasons and mechanisms explaining the shorter survival observed in patients treated with ATB and TKI are unknown. A significant component of the efficacy of sorafenib potentially resides in the capacity to improve the T-effector/T-regulatory cell ratio and augment the proportion of interferon-γ-secreting $CD8^+$ T cells.[44] Similarly, other molecularly targeted therapies such as lenvatinib[45] and cabozantinib[46] exert their pleiotropic anti-tumour effects by acting on lymphoid and myeloid constituents of the tumour microenvironment both alone and in synergy with concurrent PD-1 pathway inhibition. In addition, evidence from renal cell carcinoma suggests that VEGF pathway inhibitors can directly alter intestinal homoeostasis,[18] highlighting how ATB may plausibly alter a complex bidirectional relationship between TKI therapy and survival by acting synergistically on composition and function of the gut microbiome.

In attempting to interpret potentially causal as opposed to purely associative links between ATB and outcome, a clinically important finding from our study is the demonstration of a detrimental effect of ATB on survival observed in patients who received placebo. Although it is possible that the postulated changes in the gut microbiome and immune function mediated by ATB preconditioning could have exerted their influence even in the absence of anticancer therapy, an alternative explanation that we should consider is whether ATB dosing before treatment might select for patients with uncontrolled infection, who are at higher risk for recurrent severe infections, or who have rapidly progressive disease before treatment allocation. Patients with cirrhosis carry a higher risk of bacterial infections compared with the general population,[47] and both empirical and prophylactic ATB therapy is indicated in response to decompensation of chronic liver disease to improve survival outcomes.[48] In the absence of adequately powered translational studies evaluating the effect of ATB therapy on stool samples and peripheral immune cells, support for a causative or an associative relationship between ATB and outcome remains speculative.

Although no direct causative role between ATB and outcome can be inferred from our retrospective analysis, the utilisation of prospectively accrued, geographically heterogeneous patient cohorts, carefully selected on the basis of stringent inclusion criteria and with careful documentation of response and survival outcomes lends significant credibility to the associations observed. In addition, use of methods such as IPTW that adjust for key clinicopathologic features of cirrhosis and HCC affords sufficient robustness to our survival estimates across ATB strata and reduces the risk of bias. Among important limitations to our study are the issues associated with retrospectively examining data from completed studies. These included missing information on the number and sizes of HCC lesions, baseline AFP levels, and BCLC stages in a proportion of patients, which did not allow us to consider these variables for matching. Moreover, the restriction of ATB exposure window to 30 days might have failed to capture clinically meaningful exposures that extended beyond 30 days from treatment initiation as well as the impact of ATB treatment later during the course of systemic therapy.[20] In addition, the lack of information on indication (*i.e.* prophylactic *vs.* therapeutic), ATB class, and course length made further subgroup analyses impossible and might represent a limitation. Furthermore, we acknowledge significant heterogeneity in the studies selected for this analysis: the entirety of TKI studies included phase III trials powered on OS, whereas a number of ICI studies included phase I/II studies powered on safety outcomes and response rates, a finding that may have affected the maturity of OS and PFS data pooled for our analysis. The line of therapy was also heterogeneous across studies, and none of our OS estimates could be adjusted for post-study therapy. Finally, causes of death are not available, preventing further analyses on infection-related outcomes.

In conclusion, our study demonstrates that ATB exposure may be associated with worse PFS and OS in patients receiving ICI monotherapy as well as combinations. Although the positive association identified in our study does not prove causality, the hypothesis that an ATB-mediated gut dysbiosis may correlate with reduced antitumour efficacy is provocative and resonates with evolving clinical and translational knowledge in the field. The association between ATB exposure and outcomes from TKI therapy and placebo highlights the uniquely complex interplay between cirrhosis, cancer, risk of infection, and the pleiotropic effect of molecular therapies for HCC. To overcome the complexity of these distinctively intertwined relationships, prospective studies should investigate the role of gut microbial diversity as a determinant of outcome in patients with HCC undergoing systemic therapy. The rapid expansion of immunotherapy combinations, for which no predictive biomarker of benefit exists, highlights the importance of this stream of research as a pathway towards personalised medicine in HCC.[10]

Abbreviations

ATB, antibiotics; CTLA-4, cytotoxic T-cell lymphocyte associated antigen-4; ECOG, European Collaborative Oncology Group; HCC, hepatocellular carcinoma; HR, hazard ratio; ICI, immune checkpoint inhibitor; IPTW, inverse probability of treatment weighting; MSD, mean squared difference; OS, overall survival; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumours; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

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Conflicts of interest

DJP received lecture fees from ViiV Healthcare, Bayer Healthcare, EISAI, BMS, and Roche; travel expenses from BMS and Bayer Healthcare; and consulting fees for Mina Therapeutics, DaVolterra, Mursla, IPSEN, Exact Sciences, Avamune, EISAI, Roche, and Astra Zeneca. DJP received research funding (to institution) from MSD, GSK, and BMS. BS received travel support from AbbVie, Gilead, and Ipsen. AC received consulting fees from MSD, Astra Zeneca, Roche, and BMS. He also received speaker fees from Novartis, Astra Zeneca, and EISAI. AD received educational grant support for conference attendance by Roche. There are no other personal or financial conflicts of interest to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: DJP, LP, JS, PMK. Acquisition of data: XL, PMK, GW, JS. Analysis and interpretation of data: XL, PMK, DJP, LP, GW, AD, CAMF, BS, DRR, RP, MT, SC, SL, LF, AC. Drafting of the manuscript: DJP. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: XL, PMK. Obtained funding: DJP. Study supervision: DJP, LP.

Data availability statement

The data that support the findings of this study are available from the US FDA. Restrictions apply to the availability of these data, and employees of the FDA performed all raw data analyses.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100747.

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Author names in bold designate shared co-first authorship

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Supplemental information

Association between antibiotics and adverse oncological outcomes in patients receiving targeted or immune-based therapy for hepatocellular carcinoma

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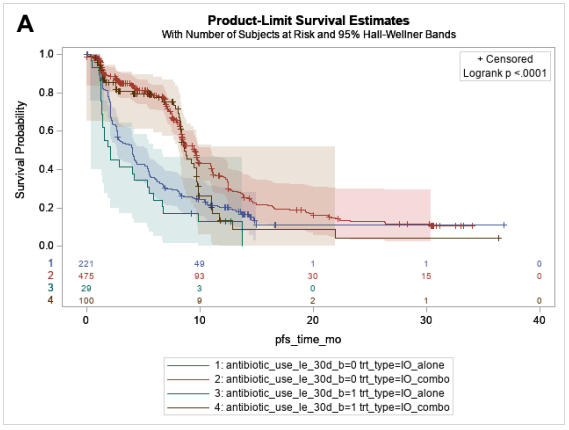

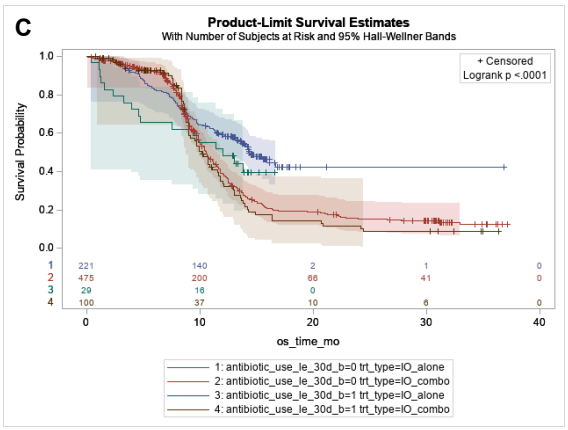

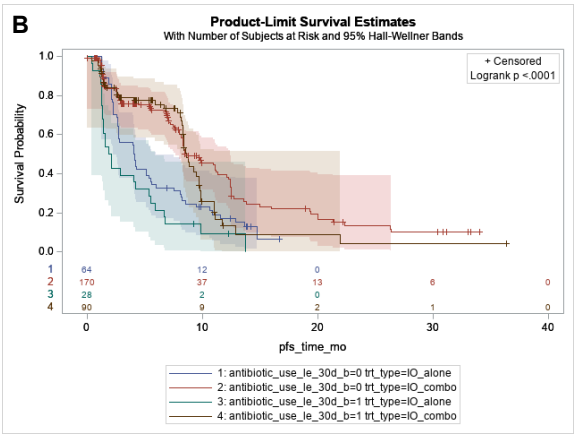

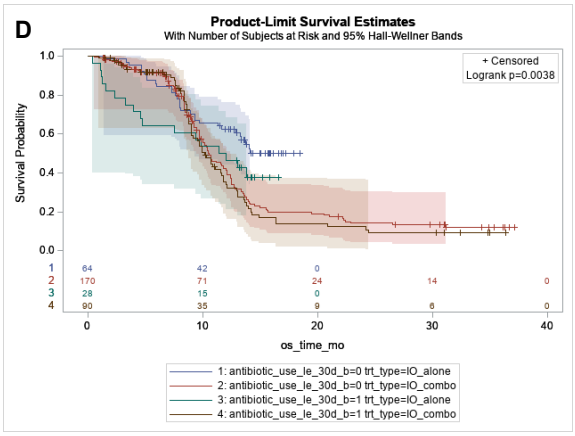

TABLES

Table S1. Use of antibiotics within 30 days of initiation of study treatment.

Antibiotic Use	Placebo (n = 808)	Tyrosine kinase inhibitor (n = 1968)	Vascular Endothelial Growth Factor (VEGF) inhibitor (n = 480)	Immunotherapy (n = 842)	All patients (n = 4098)
None or not within 30 days	708 (88%)	1656 (84%)	407 (85%)	707 (84%)	3478 (85%)
Used within 30 days	100 (12%)	312 (16%)	73 (15%)	135 (16%)	620 (15%)
Antibiotic Duration					
≤10 days of exposure	49 (49%)	140 (45%)	41 (56%)	51 (38%)	281 (45%)
>10 days of exposure	51 (51%)	172 (55%)	32 (44%)	84 (62%)	339 (55%)

Table S2. Baseline patient characteristics stratified by antibiotic exposure prior to and after inverse probability of treatment weighting (IPTW): TKI group.

	No antibiotic use N = 1640 Effect size = 1640		Antibiotic use N = 303 Effect size = 303		Mean Squared Differences before Propensity Score Weighting	No antibiotic use N = 1469 Effect size = 1746.74		Antibiotic use N = 278 Effect size = 1752.47		Mean Squared Differences before Propensity Score Weighting
Variable	**Mean/N**	**STD/%**	**Mean/N**	**STD/%**		**Mean/N**	**STD/%**	**Mean/N**	**STD/%**	
Age										
Mean/SD	61.96	11.67	62.38	11.70	0.035	61.87	12.53	61.91	29.09	0.002
Median/Range	63	19-88	63	27-88		63	19-88	62	27-88	
Race										
Asian	899	54.8	166	54.8	-0.001	977	55.9	975	55.6	-0.006
Other/Not reported	131	8.0	33	10.9	0.10	162	9.3	156	8.9	-0.01
White	610	37.2	104	34.3	-0.06	607	34.8	622	35.5	0.01
Sex										
Female	247	15.1	64	21.1	0.16	274	15.7	273	15.6	-0.003
Male	1393	84.9	239	78.9	-0.16	1473	84.3	1480	84.4	0.003
Region										
Asia	836	51.0	153	50.5	-0.01	903	51.7	905	51.7	-0.001
Rest of world	804	49.0	150	49.5	0.01	844	48.3	847	48.3	0.001
ECOG PS										
0	1010	61.6	178	58.7	-0.06	1079	61.8	1110	63.3	0.03
1	630	38.4	125	41.3	0.06	667	38.2	642	36.7	-0.03
Etiology										
Non-viral	353	21.5	68	22.4	0.02	420	24.0	427	24.3	0.01
Viral	1118	68.2	210	69.3	0.02	1327	76.0	1326	75.7	-0.01
Prior Systemic Therapy										
Yes	957	58.4	154	50.8	-0.15	917	52.5	924	52.7	0.004
No	683	41.7	149	49.2	0.15	830	47.5	828	47.3	-0.004
Macrovascular										
Absence	1224	74.6	218	71.9	-0.06	1289	73.8	1298	74.1	0.006
Presence	414	25.2	85	28.1	0.06	458	26.2	455	25.9	-0.006
Extrahepatic										
Absence	572	34.9	88	29.0	-0.13	597	34.2	606	34.6	0.01
Presence	1068	65.1	215	71.0	0.13	1150	65.8	1147	65.4	-0.01

Table S3. Baseline patient characteristics stratified by antibiotic exposure prior to and after inverse probability of treatment weighting (IPTW): immunotherapy group.

	No antibiotic use N = 696 Effect size = 696		Antibiotic use N = 129 Effect size = 129		Mean Squared Differences before Propensity Score Weighting	No antibiotic use N = 660 Effect size = 779.56		Antibiotic use N = 120 Effect size = 788.67		Mean Squared Differences before Propensity Score Weighting
Variable	**Mean/N**	**STD/%**	**Mean/N**	**STD/%**		**Mean/N**	**STD/%**	**Mean/N**	**STD/%**	
Age										
Mean/SD	62.75	11.51	63.39	11.80	0.05	63.49	11.70	62.99	11.76	-0.04
Median/Range	64	18-88	64	34-88		64	18-88	64	34-88	
Race										
Asian	337	48.4	65	50.4	0.04	397	50.9	408	51.8	0.02
Other/Not reported	35	5.0	11	8.5	0.14	45	5.7	42	5.3	-0.02
White	312	44.8	51	39.5	-0.11	338	43.3	338	42.9	-0.01
Sex										
Female	129	18.5	28	21.7	0.08	145	18.5	148	18.8	0.005
Male	567	81.5	101	78.3	-0.08	635	81.5	641	81.2	-0.005
Region										
Asia	307	44.1	53	41.1	-0.06	356	45.7	368	46.6	0.02
Rest of world	388	55.7	76	58.9	0.06	423	54.3	421	53.4	-0.02
ECOG PS										
0	434	62.4	72	55.8	-0.13	473	60.6	472	59.9	-0.015
1	262	37.6	57	44.2	0.13	307	39.4	316	40.1	0.015
Etiology										
Non-viral	222	31.9	49	38.0	0.13	255	32.7	252	32.0	-0.01
Viral	458	65.8	74	57.4	-0.17	525	67.3	537	68.0	0.01
Prior Systemic Therapy										
No	355	51.0	76	58.9	0.16	395	50.7	390	49.4	-0.03
Yes	341	49.0	53	41.1	-0.16	384	49.3	399	50.6	0.03
Immunotherapy										
Monotherapy	221	31.8	29	22.5	-0.21	240	30.9	263	33.3	0.05
Combination	475	68.2	100	77.5	0.21	539	69.1	526	66.7	-0.05
Macrovascular										
Absence	478	68.7	90	69.8	0.02	533	68.3	552	69.9	0.03
Presence	218	31.3	38	29.5	-0.04	247	31.7	237	30.1	-0.03
Extrahepatic										
Absence	236	33.9	38	29.5	-0.10	249	31.9	276	35.0	0.07
Presence	460	66.1	91	70.5	0.10	531	68.1	513	65.0	-0.07

Table S4. Baseline patient characteristics stratified by antibiotic exposure prior to and after inverse probability of treatment weighting (IPTW): placebo group.

Variable	No antibiotic use N = 697 Effect size = 697 Mean/N	STD/%	Antibiotic use N = 97 Effect size = 97 Mean/N	STD/%	Mean Squared Differences before Propensity Score Weighting	No antibiotic use N = 675 Effect size = 766.69 Mean/N	STD/%	Antibiotic use N = 92 Effect size = 722.45 Mean/N	STD/%	Mean Squared Differences before Propensity Score Weighting
Age										
Mean/STD	62.50	11.09	62.55	12.11	0.004	62.26	11.61	62.01	11.62	-0.02
Median/Range	63	23-86	63	24-85		63	23-86	63	24-85	
Race										
Asian	300	43.0	38	39.2	-0.08	335	43.7	339	43.9	0.005
Other/Not reported	69	9.9	9	9.3	-0.02	78	10.2	85	11.0	0.03
White	315	45.2	46	47.4	0.04	354	46.1	348	45.1	-0.02
Sex										
Female	89	12.8	24	24.7	0.31	106	13.8	104	13.4	-0.01
Male	608	87.2	73	75.3	-0.31	661	86.2	669	86.6	0.01
Region										
Asia	432	62.0	61	62.9	-0.02	298	38.9	309	40.0	0.02
Rest of world	418	60.0	45	46.4	0.02	469	61.1	463	60.0	-0.02
ECOG PS										
0	279	40.0	52	53.6	-0.27	452	59.0	458	59.2	0.01
1	10	1.4	1	1.0	0.27	314	41.0	315	40.8	-0.01
Etiology										
Non-viral	264	37.9	30	30.9	-0.15	286	37.3	304	39.3	0.04
Viral	423	60.7	66	68.0	0.15	480	62.7	469	60.7	-0.04
Prior Systemic Therapy										
No	0	-	0	-	-	0	-	0	-	-
Yes	697	-	97	-	-	766.69	-	772.45	-	-
Macrovascular										
Absence	489	70.2	62	63.9	-0.13	536	69.9	550	71.3	0.03
Presence	208	29.8	35	36.1	0.13	231	30.1	222	28.7	-0.03
Extrahepatic										
Absence	197	28.3	26	26.8	-0.03	214	27.9	208	26.9	-0.02
Presence	500	71.7	71	73.2	0.03	553	72.1	565	73.1	0.02

Table S5. The relationship between antibiotic use and efficacy outcomes in patients with unresectable/advanced HCC treated with immune checkpoint inhibitor (ICI) combinations or monotherapy.

	Unadjusted Analyses		Adjusted using IPTW *	
	No Antibiotic Use	**Antibiotic Use**	**No Antibiotic Use**	**Antibiotic Use**
Immune checkpoint inhibitor combinations				
Sample size				
# patients	475	100	448	92
Effect size	475	100	539.76	542.65
Progression-free survival (PFS)				
Median (95% CI), mo	9.6 (8.5, 9.8)	8.6 (8.3, 9.7)	9.7 (8.5, 11.0)	8.6 (8.3, 9.7)
HR** (95% CI)	1.33 (0.97, 1.81)		1.50 (1.26, 1.77)	
Overall survival (OS)				
Median (95% CI), mo	10.5 (10.0, 11.2)	10.2 (9.0, 11.6)	10.6 (10.2, 11.4)	9.9 (9.0, 11.6)
HR** (95% CI)	1.13 (0.87, 1.47)		1.14 (0.99, 1.32)	
Immune checkpoint inhibitor monotherapy				
Sample size				
# patients	221	29	212	28
Effect Size	221	29	240.20	228.58
Progression-free survival (PFS)				
Median (95% CI), mo	4.0 (2.8, 4.4)	2.1 (1.3, 5.4)	4.0 (2.8, 4.4)	2.1 (1.3, 5.4)
HR (95% CI)	1.51 (1.00, 2.28)		1.49 (1.22 ,1.80)	
Overall survival (OS)				
Median (95% CI), mo	14.3 (13.2, NE)	12.0 (4.6, NE)	14.4 (13.2, NE)	13.0 (4.6, NE)
HR (95% CI)	1.47 (0.88, 2.45)		1.31 (1.02, 1.68)	

** The weights were derived from a propensity score (IPTW) models which included the following baseline variables as covariates: age, race, gender, region, European Collaborative Oncology Group (ECOG) performance status, etiology of chronic liver disease, presence of macrovascular invasion, presence of extrahepatic disease and receipt of prior lines of treatment.*

*** HR is stratified by immune checkpoint inhibitor alone or immune checkpoint inhibitor combination.*

Table S6. Baseline patient characteristics stratified by antibiotic exposure prior to and after propensity score matching: Tyrosine kinase inhibitor (TKI) group.

Variable	No antibiotic use N = 1640		Antibiotic use N = 303		Mean Squared Differences before matching	No antibiotic use N = 548		Antibiotic use N = 276		Mean Squared Differences after matching
	Mean/N	STD/%	Mean/N	STD/%		Mean/N	STD/%	Mean/N	STD/%	
Age	61.96	11.67	62.38	11.70	0.035	62.26	11.61	62.01	11.62	-0.021
Race										
Asian	899	54.8	166	54.8	-0.001	296	54.0	149	54.0	-0.001
Other/Not reported	131	8.0	33	10.9	0.10	64	11.7	33	12.0	0.01
White	610	37.2	104	34.3	-0.06	188	34.3	94	34.1	-0.01
Sex										
Female	247	15.1	64	21.1	0.16	107	19.5	57	20.7	0.03
Male	1393	84.9	239	78.9	-0.16	441	80.5	219	79.3	-0.03
Region										
Asia	836	51.0	153	50.5	-0.01	271	49.5	138	50.0	0.01
Rest of world	804	49.0	150	49.5	0.01	277	50.5	138	50.0	-0.01
ECOG										
0	1010	61.6	178	58.7	-0.06	325	59.3	161	58.3	-0.02
1	630	38.4	125	41.3	0.06	223	40.7	115	41.7	0.02
Etiology										
Non-viral	353	21.5	68	22.4	0.02	135	24.6	68	24.6	0.00
Viral	1118	68.2	210	69.3	0.02	413	75.4	208	75.4	0.00
Prior Therapy										
Yes	957	58.4	154	50.8	-0.15	265	48.4	129	46.7	-0.03
No	683	41.7	149	49.2	0.15	283	51.6	147	53.3	0.03
Macrovascular										
Absence	1224	74.6	218	71.9	-0.06	396	72.3	200	72.5	0.004
Presence	414	25.2	85	28.1	0.06	152	27.7	76	27.5	-0.004
Extrahepatic										
Absence	572	34.9	88	29.0	-0.13	149	27.2	79	28.6	0.03
Presence	1068	65.1	215	71.0	0.13	399	72.8	197	71.4	-0.03

Table S7. Baseline patient characteristics stratified by antibiotic exposure prior to and after propensity score matching: immunotherapy group.

Variable	No antibiotic use N = 696		Antibiotic use N = 129		Mean Squared Differences before matching	No antibiotic use N = 234		Antibiotic use N = 118		Mean Squared Differences after matching
	Mean/N	STD/%	Mean/N	STD/%		Mean/N	STD/%	Mean/N	STD/%	
Age	62.75	11.51	63.39	11.80	0.05	63.49	11.70	62.99	11.76	-0.04
Race										
Asian	337	48.4	65	50.4	0.04	115	49.1	62	52.5	0.07
Other/Not reported	35	5.0	11	8.5	0.14	21	9.0	10	8.5	-0.02
White	312	44.8	51	39.5	-0.11	98	41.9	46	39.0	-0.06
Sex										
Female	129	18.5	28	21.7	0.08	47	20.1	24	20.3	0.006
Male	567	81.5	101	78.3	-0.08	187	79.9	94	79.7	-0.006
Region										
Asia	307	44.1	53	41.1	-0.06	95	40.6	51	43.2	0.05
Rest of world	388	55.7	76	58.9	0.06	139	59.4	67	56.8	-0.05
ECOG										
0	434	62.4	72	55.8	-0.13	133	56.8	67	56.8	-0.001
1	262	37.6	57	44.2	0.13	101	43.2	51	43.2	0.001
Etiology										
Non-viral	222	31.9	49	38.0	0.13	96	41.0	46	39.0	-0.04
Viral	458	65.8	74	57.4	-0.17	138	59.0	72	61.0	0.04
Prior Therapy										
No	355	51.0	76	58.9	0.16	122	52.1	66	55.9	0.08
Yes	341	49.0	53	41.1	-0.16	112	47.9	52	44.1	-0.08
Immunotherapy										
Monotherapy	221	31.8	29	22.5	-0.21	64	27.4	28	23.7	-0.08
Combination	475	68.2	100	77.5	0.21	170	72.6	90	76.3	0.08
Macrovascular										
Absence	478	68.7	90	69.8	0.02	158	67.5	81	68.6	0.02
Presence	218	31.3	38	29.5	-0.04	76	32.5	37	31.4	-0.02
Extrahepatic										
Absence	236	33.9	38	29.5	-0.10	64	27.4	34	28.8	0.03
Presence	460	66.1	91	70.5	0.10	170	72.6	84	71.2	-0.03

Table S8. Baseline patient characteristics stratified by antibiotic exposure prior to and after propensity score matching: placebo group.

Variable	No antibiotic use N = 697		Antibiotic use N = 97		Mean Squared Differences before matching	No antibiotic use N = 175		Antibiotic use N = 91		Mean Squared Differences after matching
	Mean/N	STD/%	Mean/N	STD/%		Mean/N	STD/%	Mean/N	STD/%	
Age	62.50	11.09	62.55	12.11	0.004	62.77	10.72	62.56	12.14	-0.018
Race										
Asian	300	43.0	38	39.2	-0.08	69	39.4	37	40.7	0.025
Other/Not reported	69	9.9	9	9.3	-0.02	21	12.0	9	9.9	-0.07
White	315	45.2	46	47.4	0.04	85	48.6	45	49.5	0.02
Sex										
Female	89	12.8	24	24.7	0.31	41	23.4	23	25.3	0.04
Male	608	87.2	73	75.3	-0.31	134	76.6	68	74.7	-0.04
Region										
Asia	432	62.0	61	62.9	-0.02	63	36.0	35	38.5	0.05
Rest of world	418	60.0	45	46.4	0.02	112	64.0	56	61.5	-0.05
ECOG										
0	279	40.0	52	53.6	-0.27	90	51.4	44	48.4	-0.06
1	10	1.4	1	1.0	0.27	85	48.6	47	51.6	0.06
Etiology										
Non-viral	264	37.9	30	30.9	-0.15	50	28.6	28	30.8	0.05
Viral	423	60.7	66	68.0	0.15	125	71.4	63	69.2	-0.05
Prior Therapy										
No	0	-	0	-	-	0	-	0	-	-
Yes	697	-	97	-	-	175	-	91	-	-
Macrovascular										
Absence	489	70.2	62	63.9	-0.13	122	69.7	59	64.8	-0.10
Presence	208	29.8	35	36.1	0.13	53	30.3	32	35.2	0.10
Extrahepatic										
Absence	197	28.3	26	26.8	-0.03	54	30.9	23	25.3	-0.12
Presence	500	71.7	71	73.2	0.03	121	69.1	68	74.7	0.12

Table 9. The relationship between antibiotic use and efficacy outcomes in patients with unresectable/advanced HCC before and after propensity score matching.

	Unadjusted Analyses		Adjusted using propensity score	
	No Antibiotic Use	**Antibiotic Use**	**No Antibiotic Use**	**Antibiotic Use**
All patients				
Sample size				
# patients	3435	601	1091	550
Progression-free survival (PFS)				
Median (95% CI), months	4.2 (4.1, 4.5)	3.6 (3.0, 4.0)	5.4 (4.3, 5.5)	3.6 (3.0, 4.0)
HR* (95% CI)	1.36 (1.23, 1.50)		1.39 (1.23 ,1.57)	
Overall survival (OS)				
Median (95% CI), months	10.8 (10.4, 11.3)	8.6 (7.9, 9.1)	11.1 (10.4, 11.8)	8.6 (7.8, 9.2)
HR* (95% CI)	1.42 (1.29, 1.57)		1.41 (1.25 ,1.59)	
Tyrosine kinase inhibitor Group				
Sample size				
# patients	1640	303	548	276
Progression-free survival (PFS)				
Median (95% CI), months	5.6 (5.5, 5.7)	3.7 (3.6, 4.3)	5.5 (4.7 ,5.6)	3.7 (3.6 ,4.3)
HR (95% CI)	1.40 (1.22, 1.61)		1.27 (1.08 ,1.51)	
Overall survival (OS)				
Median (95% CI), months	12.2 (11.6, 13.1)	8.4 (7.1, 9.8)	12.1 (10.7 ,13.9)	8.6 (7.1 ,10.1)
HR (95% CI)	1.51 (1.31, 1.73)		1.41 (1.19 ,1.68)	
Immunotherapy Group				
Sample size				
# patients	696	129	234	118
Progression-free survival (PFS)				
Median (95% CI), months	8.2 (7.6, 8.4)	8.3 (6.8, 8.9)	8.1 (7.0, 8.5)	8.3 (6.7, 8.6)
HR* (95% CI)	1.39 (1.08, 1.78)		1.41 (1.06, 1.71)	
Overall survival (OS)				
Median (95% CI), months	11.4 (10.6, 12.1)	10.7 (9.1, 11.8)	11.8 (10.4 ,12.7)	10.7 (9.1 ,11.8)
HR* (95% CI)	1.19 (0.94 ,1.50)		1.19 (0.91 ,1.57)	
Placebo Group				
Sample size				
# patients	697	97	175	91
Progression-free survival (PFS)				
Median (95% CI), months	1.87 (1.81, 1.93)	1.61 (1.41, 1.84)	1.8 (1.7 ,2.1)	1.61 (1.41 ,1.91)
HR (95% CI)	1.29 (1.03, 1.62)		1.31 (1.00 ,1.71)	
Overall survival (OS)				
Median (95% CI), months	8.2 (7.4, 8.9)	4.4 (3.7, 6.6)	6.87 (5.45 ,9.40)	4.17 (3.45 ,5.88)
HR (95% CI)	1.58 (1.25, 1.99)		1.54 (1.16 ,2.05)	

** HR is stratified by treatment group (tyrosine kinase inhibitor or immune checkpoint inhibitor alone or immune checkpoint inhibitor combination or Placebo or VEGF for all patients, immune checkpoint inhibitor alone or immune checkpoint inhibitor combination for immune checkpoint inhibitor group).*

Table S10. The relationship between antibiotic use and efficacy outcomes in patients with unresectable/advanced HCC treated with immune checkpoint inhibitor (ICI) combinations or monotherapy before and after propensity score matching.

	Unadjusted Analyses		Adjusted using propensity score	
	No Antibiotic Use	**Antibiotic Use**	**No Antibiotic Use**	**Antibiotic Use**
Immune checkpoint inhibitor combinations				
Sample size				
# patients	475	100	177	90
Progression-free survival (PFS)				
Median (95% CI), months	9.6 (8.5, 9.8)	8.6 (8.3, 9.7)	11.0 (8.4, 12.1)	8.6 (8.3, 9.7)
HR (95% CI)	1.33 (0.97, 1.81)		1.61 (1.12, 2.33)	
Overall survival (OS)				
Median (95% CI), months	10.5 (10.0, 11.2)	10.2 (9.0, 11.6)	11.4 (10.4, 12.1)	10.2 (9.0, 11.6)
HR (95% CI)	1.13 (0.87, 1.47)		1.23 (0.91, 1.68)	
Immune checkpoint inhibitor monotherapy				
Sample size				
# patients	221	29	56	28
Progression-free survival (PFS)				
Median (95% CI), months	4.0 (2.8, 4.4)	2.1 (1.3, 5.4)	4.0 (2.6, 5.4)	2.1 (1.3, 4.2)
HR (95% CI)	1.51 (1.00, 2.28)		1.48 (0.92, 2.38)	
Overall survival (OS)				
Median (95% CI), months	14.3 (13.2, NE)	12.0 (4.6, NE)	14.4 (10.5, NE)	11.9 (4.6, NE)
HR (95% CI)	1.47 (0.88, 2.45)		1.61 (0.87, 2.97)	

NE = not estimable