Associations between dysbiosis-inducing drugs, overall survival and tumor response in patients treated with immune checkpoint inhibitors

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Abstract

Background: There are conflicting data on the effects of dysbiosis-inducing drugs, and especially antibiotics (ATBs), on clinical outcomes in patients treated with immune checkpoint inhibitors (ICIs). There is a particular lack of data for patients with melanoma.

Methods: We performed a single-center retrospective study of the associations between ATBs and other drugs known to modify the gut microbiota (proton pump inhibitors, nonsteroidal anti-inflammatory drugs, statins, opioids, anti-vitamin K, levothyroxine, vitamin D3, antiarrhythmics, metformin and phloroglucinol), overall survival (OS) and tumor response in consecutive cancer patients (particularly those with melanoma) treated with an ICI (ipilimumab, nivolumab or pembrolizumab) over a 9-year period.

Results: A total of 372 patients were included. The mean ± standard deviation age was 64.0 ± 12.1 years. The most frequently prescribed ICI was nivolumab (in 58.3% of patients) and the most frequent indications were lung cancer (44.6%) and melanoma (29.6%). Overall, 112 patients (30.1%) had received ATBs. ATB use was associated with (1) shorter OS in the study population as a whole [adjusted hazard ratio [95% confidence interval (CI)]: 1.38 (1.00–1.90), $p = 0.048$] and in patients with melanoma [adjusted hazard ratio (95% CI): 2.60 (1.06–6.39), $p = 0.037$], and (2) a lower response rate in the study population as a whole [8.1%, *versus* 31.1% in patients not treated with ATBs; adjusted odds ratio (95% CI): 6.06 (2.80–14.53), $p < 0.001$] and in patients with melanoma [adjusted odds ratio (95% CI): 4.41 (1.04–22.80), $p = 0.045$]. Sensitivity analyses that minimized the indication bias did not reveal an association between OS and the presence of an infection requiring ATBs (quantified as the severity of infection, hospitalization for an infection, or ICI discontinuation). Other dysbiosis-inducing drugs were not associated with a difference in OS.

Conclusion: Unlike other dysbiosis-inducing drugs, ATBs were associated with poorer clinical outcomes in ICI-treated patients overall and in the subset of patients with melanoma.

Keywords: antibiotic, drug-induced dysbiosis, dysbiosis, immune checkpoint inhibitor, melanoma

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Introduction

In the early 2000s, the advent of immune checkpoint inhibitors (ICIs) led to a new era in cancer treatment. By targeting inhibitory receptors (CTLA-4 and PD-1) or a ligand (PD-L1) expressed on the surface of lymphocytes,[1,2] ICIs promote the immune-mediated control of tumor growth. The use of ICIs by patients with skin, lung, kidney, ear nose and throat and hematological cancers has led to marked reductions in morbidity

and mortality.[3–5] However, tumor escape is still a major problem.

According to the literature, between 60% and 80% of patients fail to respond to ICIs.[6] The factors underlying escape from ICIs can be divided into three classes: tumor-related factors, host factors, and environmental factors.[7] The latter include infectious agents, diet, prolonged exposure to the sun, concomitant drug treatments (including exposure to corticosteroids and the indications requiring treatment with corticosteroids),[8] and the gut microbiota (i.e. all the microorganisms in the digestive tract, primarily bacteria but also viruses, fungi, and archaea). Interaction with the microbiota (mainly the bacteria) influences the host's digestive, metabolic, neurological and immunological functions.[9]

A number of studies have highlighted a link between dysbiosis (i.e. a quantitative and/or qualitative imbalance in the microbiota) and the patient's response to ICIs.[10,11] For example, the treatment success rate is directly related to the proportions of certain bacteria.[12] Thus, anti-CTLA-4 activity is directly related to the presence of certain Bacteroidetes (the Bacteroidales) and Proteobacteria (the Burkholderiales).[13] In fact, an increase in the proportion of these bacteria seems to be associated with greater effectiveness of ipilimumab.[14] Similarly, anti-PD-1 activity is modulated by the presence of the order Bacteroidales (the genus *Bacteroides*), the genus *Bifidobacterium*, the phylum Actinobacteria, *Akkermansia muciniphilia*, the phylum Verrucomicrobia, and the phylum Firmicutes.[10,13] Other studies have highlighted an association between anti-PD-L1 activity and *Bifidobacterium*, Actinobacteria, *Collinsella aerofaciens*, and *Enterococcus faecium*.[13]

Drugs that influence the composition of the gut microbiota [e.g. antibiotics (ATBs)] can influence the clinical effectiveness of drugs. To date, studies of the effectiveness of ICIs have not considered the impact of individual ATBs on the gut microbiota.[10,15–21] In fact, it is known that the impact on the microbiome differs from one ATB to another. For example, treatments with ceftriaxone and ofloxacin tend to increase and reduce the proportion of *Enterococcus*, respectively.[13] The duration of dysbiosis after ATB discontinuation also differs from one compound to another. Thus, spiramycin seems to affect the microbiota for up to 28 days after discontinuation, whereas piperacillin has an effect for up to 14 days.[13] Hence, the change in the microbiota induced by a particular ATB can influence the interaction with ICIs. For example, the use of amoxicillin and amoxicillin/clavulanic acid might boost the effectiveness of ICIs by increasing the number of *Bacteroides*.[13]

Retrospective studies of the association between ATB use and overall survival (OS) in patients treated with ICIs have generated conflicting results: some have shown a negative association,[16,17,19,22] while others did not observe an association.[15,20,21] As described by Derosa *et al.*,[22] the timeframe of exposure to ATBs before ICI initiation appears to be a determinant factor; the clinical outcomes in patients exposed to ATBs 60 days before ICI initiation were better than in patients exposed for just 30 days before initiation. Furthermore, most of these studies focused on patients with renal cell carcinoma, urothelial carcinoma (UC) or nonsmall-cell lung carcinoma (NSCLC) but not on those with melanoma. Moreover, the researchers did not determine whether the severity of the infection influenced the association between ATB use and worse OS. Lastly, recent *post hoc* analyses of randomized trials of patients with UC and NSCLC showed worse OS and progression-free survival in individuals in the ICI arm (the anti-PD-L1 atezolizumab) receiving ATBs but not in those in the control arm (conventional chemotherapy) receiving ATBs.[23,24]

Along with ATBs, a number of other drug classes can induce dysbiosis: proton pump inhibitors (PPIs), drugs for functional gastrointestinal disorders (particularly phloroglucinol), anti-vitamin K (AVK) anticoagulants, antiarrhythmics, nonsteroidal anti-inflammatory drugs (NSAIDs), vitamin D3 (known for its protective role in intestinal homeostasis),[25] metformin (which stimulates the gut microbiota and the immune system),[26] opioids, statins (which appear to be associated with an anti-inflammatory gut microbiotic profile),[27] levothyroxine, and psychotropics.[28–35]

The objective of the present study was to evaluate the associations between ATBs (especially amoxicillin and amoxicillin/clavulanic acid) and other drugs known to modify gut microbiota on one hand and OS and the tumor response in patients treated with ICIs (particularly patients with melanoma) on the other.

Methods

Study design

We performed a retrospective, observational study of all consecutive adult patients (aged 18 years and over) treated with an anti-CTLA-4 agent (ipilimumab) and/or an anti-PD-1 agent (nivolumab or pembrolizumab) in the departments of oncology, dermatology, pulmonology, hematology and gastroenterology from December 2010 to December 2019 at Amiens University Medical Center (Amiens, France). Patients enrolled in clinical trials or receiving concomitant chemotherapy or targeted therapy were not included.

Collection of patients' baseline characteristics

Demographic characteristics (age and sex), body mass index (BMI), and comorbidities were collected: smoking status (defined as 'never' or 'current/past'), alcohol consumption (defined as daily consumption or not, regardless of the dose), a history of cardiovascular disease (including myocardial infarction, stroke, obliterating arteriopathy of the lower limbs, and deep vein thrombosis), the presence or absence of diabetes mellitus (regardless of the severity), the presence or absence of high blood pressure, the presence or absence of dyslipidemia, and a history of cancer. The type of current cancer, its metastatic status, the number of metastatic sites (including brain metastases, if present), and the Eastern Cooperative Oncology Group (ECOG) performance status were collected. Any conventional and targeted chemotherapies prior to ICI initiation were recorded. The first-line treatment of ICIs was considered in the analyses, and subsequent lines (if applicable) were also described.

Evaluation of the tumor response and overall survival

On the basis of data gathered from multidisciplinary team meeting reports and imaging reports, the best overall response was defined as a complete response (CR), partial response (PR), stable disease (SD) or disease progression (PD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.1).[36] A good response was defined as CR or PR status. OS was calculated from the date of ICI initiation to the time of death from any cause or to the date of the last follow-up examination.

Collection of data on drug use and definitions of patient groups

Antibiotics. When defining the patient groups, we took account of the dysbiosis caused by each ATB,[13] the influence of dysbiosis on the ICI's effectiveness[10,14,37,38] and the time needed for recovery of the gut microbiota after ATB discontinuation.[13] Patients with a documented tumor response at least 3 months after the initiation of ICI treatment were assigned to the 'ATB+' group if they had received amoxicillin in the year preceding ICI initiation, amoxicillin/clavulanic acid in the preceding 60 days, piperacillin–tazobactam, cloxacillin or oracillin in the preceding 10 days, any cephalosporin in the preceding 40 days, any macrolide in the preceding 28 days, vancomycin (only for patients treated with an anti-PD-1 agent) in the preceding 7 days, any quinolone in the preceding year, metronidazole in the preceding 60 days, doxycycline in the preceding 7 days, sulfamethoxazole–trimethoprim in the preceding 14 days, linezolid in the preceding 28 days, any aminoglycoside in the preceding 30 days, or any type of ATB in the 60 days following the initiation of ICI treatment. The ATB's administration route was documented, along with the treatment duration, the indication, the severity of the confirmed or suspected infection (classified as systemic inflammatory response syndrome, sepsis, severe sepsis, septic shock,[39] or a nonsystemic infection), hospitalization prompted by an infection, and ICI discontinuation prompted by an infection.

Other dysbiosis-inducing drugs. Given the lack of literature data on the gut microbiota's recovery after the discontinuation of PPIs, NSAIDs, statins, metformin, opioids, cholecalciferol, levothyroxine, AVK anticoagulants, antiarrhythmic, phloroglucinol and psychotropics, patients were assigned to the corresponding drug class group if they received this drug class upon initiation of ICI treatment or in the following 60 days. For each drug class, the indication was documented.

Steroids. Given that systemic treatment with corticosteroids is known to lower the effectiveness of ICIs,[40–42] the use of oral corticosteroids in the 60 days following the initiation of ICI treatment, together with the indication [an immune-related adverse event (irAE) or another indication], and the dose level were documented.

Ethical approval and informed consent statements

In line with the French legislation on retrospective analyses of routine clinical practice, patients were not required to give their informed consent. On admission to hospital, however, patients could refuse the use of their medical data for research purposes. The present study protocol was approved by an institutional committee with competency for studies not requiring approval by an independent ethics committee (Clinical Research Directorate, Amiens University Medical Center, Amiens, France) and was registered with the French National Data Protection Commission (Commission Nationale de l'Informatique et des Libertés, Paris, France; reference: PI2018_843_0062, dated 11 November 2018).

Statistical analyses

In our descriptive analysis, categorical variables were expressed as the number (percentage). Continuous variables were expressed as the mean ± standard deviation (SD), the median (interquartile range), or the median (range), depending on the data distribution. In a bivariate analysis comparing patients receiving the drug of interest with those not receiving it, continuous variables were compared using Student's *t* test or a Wilcoxon's rank-sum test (depending on the data distribution), and categorical variables were compared using a Chi-square test or Fisher's exact test. OS in the ATB+ and ATB− groups was assessed using the Kaplan–Meier method and a log-rank test. The groups' tumor response rates (according to the RECIST 1.1 criteria) were also compared. In multivariate analyses, a Cox proportional hazards model was built for OS and a logistic regression model was built for the tumor response (with a worse tumor response defined as SD or PD status). Variables with a p-value < 0.2 in the bivariate analysis and the predictors of death most frequently described in the literature (i.e. age, sex, BMI, current or past smoking, alcohol consumption, a history of cardiovascular disease, cancer duration, and ECOG performance status) were included in the models, together with other drugs associated with better or poorer OS or a better or worse tumor response in bivariate analyses. A Cox proportional hazards model and a logistic regression including diabetes, hypertension, dyslipidemia, number of metastatic sites (as a binary variable: at least two metastatic sites *versus* fewer than two metastatic sites) and the line of treatment of ICI were also applied.

In sensitivity analyses, the same analyses were performed in (1) patients exposed to ATBs in the 30 days preceding or following the initiation of ICI (the 'ATB+30' group) *versus* those who were not (the 'ATB-30' group) and (2) patients exposed to ATBs in the 60 days preceding or following the initiation of ICI (the 'ATB+60' group) *versus* those who were not (the 'ATB-60' group).

We also analyzed the subsets of patients in the ATB+ and ATB− groups with melanoma. For these subgroups, prognosis factors [i.e. brain metastasis, levels of lactate dehydrogenase (LDH) known at ICI initiation, and ulceration as a histological characteristic of the melanoma][43] were included in the multivariate analyses (Cox proportional hazards model and logistic regression).

Moreover, we evaluated OS and the tumor response in patients having received amoxicillin and/or amoxicillin/clavulanic acid *versus* patients having received other ATBs and patients having not received ATBs. In order to minimize indication bias, several sensitivity analyses were performed: (1) patients who died or were lost to follow-up within 4 weeks of ATB discontinuation; (2) OS in patients who discontinued the ICI due to an infection *versus* those who did not, (3) OS in hospitalized patients requiring systemic ATB therapy *versus* patients not hospitalized, and (4) OS in patients with a clinical infection *versus* those with sepsis.

All statistical tests were two-sided, and the threshold for statistical significance was set to $p < 0.05$. All analyses were performed using R software (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 374 patients started treatment with ipilimumab, nivolumab or pembrolizumab (alone or in combination) between 1 December 2010, and 31 December 2019. Overall, 2 patients were excluded because of missing data and so 372 patients were included in the analysis. The mean ± SD age was 64.0 ± 12.1 years. The most frequently prescribed ICI [in 217 patients (58.3%)] was nivolumab. The most frequent cancers were lung cancers (44.6%) and melanoma (29.6%). The study population's demographic and clinicopathological characteristics are summarized in Table 1.

Table 1. Characteristics of the overall study population and the ATB– and ATB+ groups.

	Overall population *n* = 372	**ATB– group *n* = 260**	**ATB+ group *n* = 112**	***p*-value**
Age (years), mean ± SD	64.0 ± 12.1	64.8 ± 12.0	62.1 ± 12.4	0.048
Sex				
Male, *n* (%)	244 (65.6)	162 (62.3)	82 (73.2)	0.055
Female, *n* (%)	128 (34.4)	98 (37.7)	30 (26.8)	
Body mass index (kg/m²), mean ± SD	24.8 ± 5.4	25.3 ± 5.6	23.7 ± 5.0	0.009
Smoking (current or past), *n* (%)	268 (72.0)	177 (68.1)	91 (81.2)	0.013
Alcohol consumption, *n* (%)	125 (33.6)	73 (28.1)	52 (46.4)	<0.001
Cardiovascular history, *n* (%)	103 (27.7)	71 (27.3)	32 (28.6)	0.902
Diabetes mellitus, *n* (%)	51 (13.7)	38 (14.6)	13 (11.6)	0.542
High blood pressure, *n* (%)	171 (46.0)	126 (48.5)	45 (40.2)	0.175
Dyslipidemia, *n* (%)	104 (28.0)	77 (29.6)	27 (24.1)	0.337
History of cancer, *n* (%)	67 (18.0)	42 (16.2)	25 (22.3)	0.203
Tumor type				
Lung, *n* (%)	166 (44.6)	104 (40.0)	62 (55.4)	0.009
Melanoma, *n* (%)	110 (29.6)	94 (36.2)	16 (14.3)	<0.001
Renal and urothelial, *n* (%)	27 (7.3)	24 (9.2)	3 (2.7)	0.028
Head and neck, *n* (%)	48 (12.9)	25 (9.6)	23 (20.5)	0.006
Hodgkin lymphoma, *n* (%)	5 (1.3)	1 (0.4)	4 (3.6)	0.030
Digestive, *n* (%)	4 (1.1)	3 (1.2)	1 (0.9)	1.000
Cutaneous squamous cell carcinoma, *n* (%)	1 (0.3)	0	1 (0.9)	0.301
Adenocarcinoma of unknown primary, *n* (%)	5 (1.3)	5 (1.9)	0	0.328
Squamous cell carcinoma of unknown primary, *n* (%)	5 (1.3)	4 (1.5)	1 (0.9)	1.000
Porocarcinoma, *n* (%)	1 (0.3)	0	1 (0.9)	0.301
Metastatic cancer, *n* (%)	276 (74.2)	211 (81.2)	65 (58.0)	<0.001
Number of metastatic sites, med (IQR)	1 (0–2)	1 (1–2)	1 (0–2)	<0.001
Brain metastasis, *n* (%)	29 (7.8)	21 (8.1)	8 (7.1)	0.922
Cancer duration (months), med (IQR)	13.7 (6.9–33.3)	16.0 (7.6–38.8)	10.2 (5.2–19.5)	0.001
ECOG performance status				
0–1, *n* (%)	295 (79.3)	208 (80.0)	87 (77.7)	0.676
2–4, *n* (%)	77 (20.7)	52 (20.0)	25 (22.3)	
Prior conventional chemotherapy, *n* (%)	219 (58.9)	139 (53.5)	80 (71.4)	0.002
Number of lines, med (IQR)	1 (1–2)	1 (1–2)	1 (1–1)	0.289

(Continued)

Table 1. (Continued)

	Overall population *n* = 372	ATB– group *n* = 260	ATB+ group *n* = 112	*p*-value
Prior targeted chemotherapy, *n* (%)	66 (17.7)	53 (20.4)	13 (11.6)	0.059
Number of lines, med (IQR)	1 (1–1)	1 (1–2)	1 (1–1)	0.135
ICIs				
First-line treatment, *n* (%)	372 (100)	260 (100)	112 (100)	1.000
Nivolumab alone, *n* (%)	217 (58.3)	143 (55.0)	74 (66.1)	0.062
Pembrolizumab, *n* (%)	130 (34.9)	96 (36.9)	34 (30.4)	0.271
Ipilimumab alone, *n* (%)	15 (4.0)	14 (5.4)	1 (0.9)	0.046
Nivolumab + ipilimumab, *n* (%)	10 (2.7)	7 (2.7)	3 (2.7)	1.000
Second-line treatment, *n* (%)	27 (7.3)	24 (9.2)	3 (2.7)	0.028
Nivolumab, *n* (%)/*n*' = 27	11 (40.8)	9 (37.5)	2 (66.7)	0.516
Pembrolizumab, *n* (%)/*n*' = 27	7 (25.9)	6 (25.0)	1 (33.3)	0.680
Ipilimumab, *n* (%)/*n*' = 27	9 (33.3)	9 (37.5)	0	0.062
Third-line treatment, *n* (%)	2 (0.5)	2 (0.8)	0	1.000
Nivolumab, *n* (%)/*n*' = 2	0	0	0	–
Pembrolizumab, *n* (%)/*n*' = 2	1 (50.0)	1 (50.0)	0	–
Ipilimumab, *n* (%)/*n*' = 2	1 (50.0)	1 (50.0)	0	–
Factors modifying the gut microbiota				
Concomitant medications				
NSAIDs, *n* (%)	23 (6.2)	18 (6.9)	5 (4.5)	0.504
PPIs, *n* (%)	149 (40.1)	103 (39.6)	46 (41.1)	0.883
Statins, *n* (%)	83 (22.3)	58 (22.3)	25 (22.3)	1.000
Opioids, *n* (%)	173 (46.5)	119 (45.8)	54 (48.2)	0.749
Metformin, *n* (%)	17 (4.6)	16 (6.2)	1 (0.9)	0.050
AVKs, *n* (%)	16 (4.3)	13 (5.0)	3 (2.7)	0.463
Levothyroxine, *n* (%)	40 (10.8)	36 (13.8)	4 (3.6)	0.006
Cholecalciferol, *n* (%)	59 (15.9)	47 (18.1)	12 (10.7)	0.103
Phloroglucinol, *n* (%)	19 (5.1)	13 (5.0)	6 (5.4)	1.000
Antiarrhythmic drug, *n* (%)	20 (5.4)	11 (4.2)	9 (8.0)	0.214
Corticosteroids, *n* (%)	77 (20.7)	57 (21.9)	20 (17.9)	0.454
Use of food supplements, *n* (%)	58 (15.6)	27 (10.4)	31 (27.7)	<0.001

ATB, antibiotic; AVK, anti-vitamin K; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; IQR, interquartile range; NSAID: nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor; SD standard deviation.

Overall survival and tumor response as a function of concomitant medications

Antibiotics

The study population as a whole. Overall, 112 patients (30.1%) were exposed to ATBs, with intravenous administration in 39 patients (34.8%) and oral administration in 73 (65.2%). The most frequently prescribed first-line drug class was penicillin ($n = 75$, 70.0%). A total of 36 patients (32.1%) received two courses of ATB for the same infection or for separate infections, 10 patients (8.9%) received three courses, and 1 patient (2.0%) received five courses. In 88 of the patients (78.6%), ATBs were prescribed for a pre-existing infection. There were 47 cases of nonsystemic infection (42.0%), 25 cases of systemic inflammatory response syndrome (22.3%), 10 cases of sepsis (8.9%), 3 cases of severe sepsis (2.7%), and 2 cases of septic shock (1.8%). The median (interquartile range) cumulative duration of all ATB treatments for all lines was 14.50 (7.75–30.50) days. A total of 44 of the 88 patients with a pre-existing infection (50.0%) had been hospitalized for that reason (Table 2).

The ATB+ and ATB− groups differed with regard to several prognostic factors at baseline. Patients in the ATB+ group were younger than those in the ATB− group (mean ± SD age: 62.1 ± 12.4 *versus* 64.8 ± 12.0 years, respectively; $p = 0.048$). Relative to the ATB− group, the ATB+ group had a higher proportion of patients with alcohol consumption (28.1% *versus* 46.4%, respectively; $p < 0.001$) and a higher proportion of patients with lung cancer (40.0% *versus* 55.4%, respectively; $p = 0.009$). However, the proportion of patients with metastatic cancer was higher in the ATB− group than in the ATB+ group (81.2% *versus* 58.0%, respectively; $p < 0.001$), and the duration of cancer was longer in the ATB− group than in the ATB+ group [median (interquartile range): 16.0 (7.6–38.8) *versus* 10.2 (5.2–19.5) months respectively; $p = 0.001$, Table 1]. With regard to factors that could have modified the gut microbiota, the two groups were well balanced for the use of medications other than ATBs, except for levothyroxine (3.6% in the ATB− group *versus* 13.8% in the ATB+ group; $p = 0.006$, Table 1). Furthermore, the use of food supplements was more frequently reported in the ATB+ group than in the ATB− group (27.7% *versus* 10.4%, respectively; $p < 0.001$, Table 1).

Of the 112 patients in the ATB+ group and the 260 patients in the ATB− group, respectively 81 (72.3%) and 146 (56.2%) died during the study period. The median (95% CI) OS time was significantly lower in the ATB+ group than in the ATB− group [39.3 (30.9–54.1) *versus* 96.9 (64.1–152.4) weeks, respectively; crude hazard ratio (HR) (95% CI): 1.75 (1.33–2.30); $p < 0.001$ in a log-rank test; Figure 1]. The duration of ATB administration did not influence OS [median (95% CI) OS time: 39.8 (30.9–71.1) weeks in patients with an ATB duration <14.5 days *versus* 39.3 (26.0–56.9) weeks in patients with an ATB duration >14.5 days; crude HR (95% CI): 0.99 (0.64–1.53), $p = 1.000$ in a log-rank test, Supplementary Figure S1]. Intravenous ATB administration was associated with poorer OS than oral ATB administration [median (95% CI) OS time: 31.1 (21.7–53.6) *versus* 42.7 (33.1–75.3) respectively; crude HR (95% CI): 1.59 (1.02–2.49), $p = 0.040$ in a log-rank test, Supplementary Figure S2]. The results of the multivariate analysis confirmed these trends; OS was significantly shorter in the ATB+ group than in the ATB− group [adjusted HR (95% CI): 1.38 (1.00–1.90); $p = 0.048$]. Furthermore, a higher ECOG performance status was associated with a shorter survival time [adjusted HR (95% CI): 2.73 (1.92–3.89), $p < 0.001$], as was the use of corticosteroids for an indication other than an irAE [adjusted HR (95% CI): 1.33 (1.04–2.19), $p = 0.046$] but not for an irAE [adjusted HR (95% CI): 1.03 (0.55–1.94), $p = 0.926$, Table 3].

The proportion of responders was higher in the ATB− group than in the ATB+ group (33.1% *versus* 8.1% respectively; $p < 0.001$, Figure 2). After adjustment for confounding variables, the logistic regression analysis confirmed this trend [adjusted OR (95% CI): 6.06 (2.80–14.53), $p < 0.001$]. Furthermore, a higher ECOG performance status was associated with a worse tumor response [adjusted OR (95% CI): 2.71 (1.17–6.93), $p = 0.026$], as was the use of corticosteroids for an indication other than an irAE [adjusted OR (95% CI): 1.90 (1.03–5.45), $p = 0.048$] but not for an irAE [adjusted HR (95% CI): 1.57 (0.48–6.05), $p = 0.480$, Table 4].

Regarding ATB use, the results were similar when adding the variables diabetes mellitus, hypertension, dyslipidemia, the number of metastatic sites and the number of lines of ICI treatment to the models [adjusted HR (95% CI): 1.34 (1.05–1.85), $p = 0.045$ for OS, Supplementary Table S1; adjusted OR (95% CI): 5.97 (2.70–14.57), $p < 0.001$ for the tumor response, Supplementary Table S2].

Table 2. Description of infections and ATBs.

	ATB therapy group *n* = 112
Indication for ATBs	
Infection site	
Lungs, *n* (%)	46 (41.1)
Skin, *n* (%)	9 (8.0)
Oropharyngeal tract or dental, *n* (%)	4 (3.6)
Urinary tract, *n* (%)	7 (6.2)
Digestive tract, *n* (%)	3 (2.7)
Osteitis, *n* (%)	1 (0.9)
Sepsis, *n* (%)	18 (16.1)
Prophylactic ATB therapy, *n* (%)	4 (3.6)
Unknown indication, *n* (%)	20 (17.9)
Severity of infection	
Nonsystemic infection, *n* (%)	47 (42.0)
SIRS, *n* (%)	25 (22.3)
Sepsis, *n* (%)	10 (8.9)
Severe sepsis, *n* (%)	3 (2.7)
Septic shock, *n* (%)	2 (1.8)
Hospitalization due to infection, *n* (%)	44 (39.3)
Intensive care, *n* (%)	3 (2.7)
ATBs	
Administration route	
Intravenous, *n* (%)	39 (34.8)
Oral, *n* (%)	73 (65.2)
First-line treatment	*n* = *112*
Amoxicillin, *n* (%)	10 (8.9)
Amoxicillin + metronidazole, *n* (%)	1 (0.9)
Amoxicillin + clarithromycin + metronidazole, *n* (%)	1 (0.9)
Amoxicillin/clavulanic acid, *n* (%)	51 (45.5)
Amoxicillin/clavulanic acid + ciprofloxacin, *n* (%)	1 (0.9)
Amoxicillin/clavulanic acid + metronidazole, *n* (%)	1 (0.9)
Azithromycin, *n* (%)	1 (0.9)
Cefazolin, *n* (%)	1 (0.9)
Cefpodoxime, *n* (%)	1 (0.9)
Ceftazidime + ciprofloxacin, *n* (%)	1 (0.9)

(Continued)

Table 2. (Continued)

	ATB therapy group *n* = 112
Ceftriaxone, *n* (%)	4 (3.7)
Ceftriaxone + metronidazole, *n* (%)	3 (2.8)
Ceftriaxone + spiramycin, *n* (%)	1 (0.9)
Cefuroxime, *n* (%)	3 (2.8)
Ciprofloxacin, *n* (%)	1 (0.9)
Ciprofloxacin + clindamycin, *n* (%)	1 (0.9)
Ciprofloxacin + piperacillin/tazobactam, *n* (%)	1 (0.9)
Cotrimoxazole, *n* (%)	3 (2.8)
Daptomycin, *n* (%)	1 (0.9)
Daptomycin + cefepime, *n* (%)	1 (0.9)
Doxycycline, *n* (%)	5 (4.5)
Doxycycline + levofloxacin, *n* (%)	1 (0.9)
Imipenem/cilastatin + amikacin, *n* (%)	1 (0.9)
Levofloxacin, *n* (%)	2 (1.8)
Metronidazole, *n* (%)	1 (0.9)
Piperacillin/tazobactam, *n* (%)	4 (3.7)
Piperacillin/tazobactam + amikacin, *n* (%)	2 (1.8)
Piperacillin/tazobactam + amikacin + spiramycin, *n* (%)	1 (0.9)
Piperacillin/tazobactam + gentamicin + daptomycin, *n* (%)	1 (0.9)
Piperacillin/tazobactam + gentamicin + vancomycin, *n* (%)	1 (0.9)
Pristinamycin, *n* (%)	3 (2.8)
Roxithromycin, *n* (%)	1 (0.9)
Second-line treatment	*n* = *36*
Amoxicillin, *n* (%)	2 (5.5)
Amoxicillin/clavulanic acid, *n* (%)	11 (30.5)
Amoxicillin/clavulanic acid + vancomycin, *n* (%)	1 (2.8)
Cefepime, *n* (%)	1 (2.8)
Cefixime, *n* (%)	1 (2.8)
Ceftazidime, *n* (%)	1 (2.8)
Ceftriaxone, *n* (%)	1 (2.8)
Ceftriaxone + metronidazole, *n* (%)	1 (2.8)
Ciprofloxacin, *n* (%)	3 (8.2)

(Continued)

Table 2. (Continued)

	ATB therapy group *n* = 112
Ciprofloxacin + metronidazole, *n* (%)	1 (2.8)
Cotrimoxazole, *n* (%)	1 (2.8)
Imipenem/cilastatin + amikacin, *n* (%)	1 (2.8)
Linezolid, *n* (%)	2 (5.5)
Linezolid + amoxicillin, *n* (%)	1 (2.8)
Linezolid + imipenem/cilastatin	1 (2.8)
Metronidazole, *n* (%)	2 (5.5)
Piperacillin/tazobactam, *n* (%)	1 (2.8)
Piperacillin/tazobactam + ciprofloxacin, *n* (%)	1 (2.8)
Roxithromycin, *n* (%)	1 (2.8)
Telithromycin, *n* (%)	1 (2.8)
Vancomycin, *n* (%)	1 (2.8)
Third-line treatment	*n* = 10
Amoxicillin/clavulanic acid, *n* (%)	1 (10.0)
Ceftozolane/tazobactam + cefepime, *n* (%)	1 (10.0)
Ceftriaxone, *n* (%)	1 (10.0)
Ceftriaxone + spiramycin, *n* (%)	1 (10.0)
Gentamicin, *n* (%)	1 (10.0)
Piperacillin/tazobactam, *n* (%)	2 (20.0)
Piperacillin/tazobactam + ciprofloxacin, *n* (%)	1 (10.0)
Roxithromycin, *n* (%)	1 (10.0)
Spiramycin	1 (10.0)
Fourth-line treatment	*n* = 1
Ceftriaxone + spiramycin, *n* (%)	1 (100)
Fifth-line treatment	*n* = 1
Amikacin + ceftazidime, *n* (%)	1 (100)
Discontinuation of ICI due to infection, *n* (%)	24 (21.4)
Time between infection and death or loss to follow-up	*n* = 88
<4 weeks, *n* (%)	9 (10.2)
>4 weeks, *n* (%)	79 (89.8)

ATB, antibiotic; ICI, immune checkpoint inhibitor; SIRS, systemic inflammatory response syndrome.

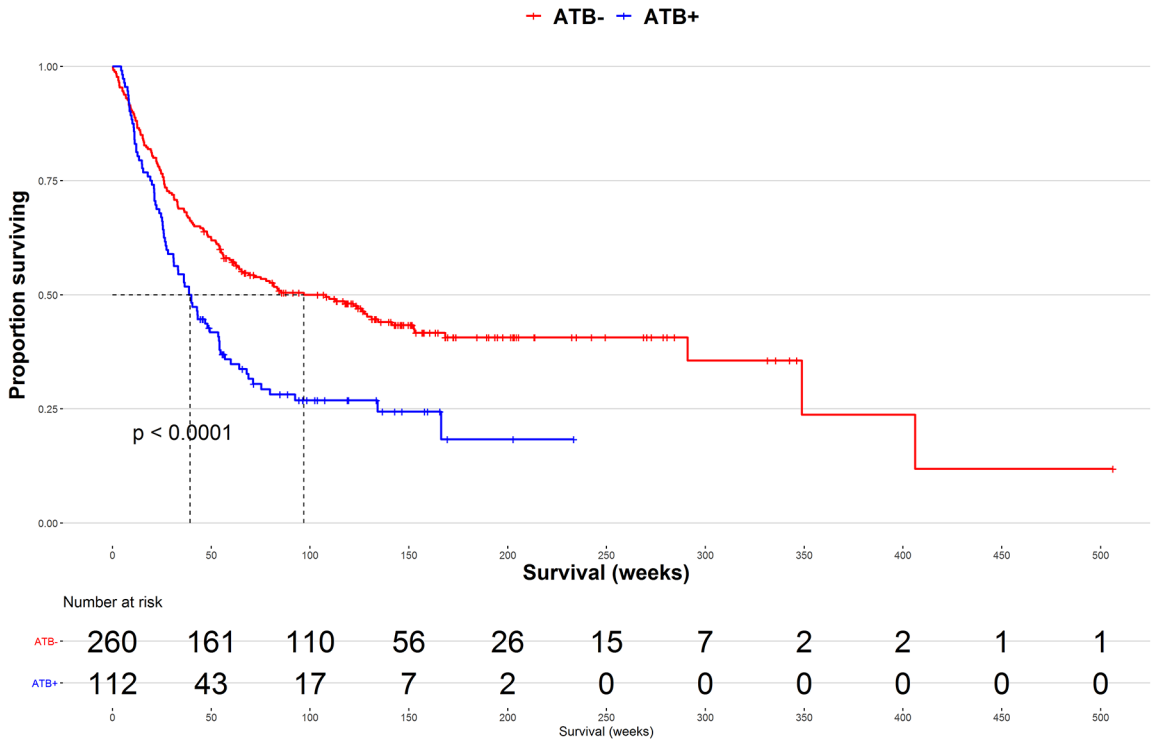

Figure 1. OS as a function of ATB use (the ATB− group *versus* the ATB+ group).
ATB, antibiotic; OS, overall survival.

The results of sensitivity analyses of patients exposed to ATBs in the 30 or 60 days preceding or following ICI initiation confirmed these trends. The median OS time was shorter (1) in the ATB+30 group than in the ATB−30 group [36.1 (26.0–46.9) *versus* 83.3 (63.6–131.1) respectively, crude HR (95% CI): 1.87 (1.38–2.53), $p < 0.001$ in a log-rank test, Supplementary Figure S3(a)] and (2) in the ATB+60 group than in the ATB−60 group [37.6 (27.3–49.3) *versus* 96.9 (63.6–141.4) weeks respectively; crude HR (95% CI): 1.79 (1.35–2.37), $p < 0.001$ in a log-rank test, Supplementary Figure S3(b)]. In Cox proportional hazards models, ATB exposure within 30 days was still associated with poorer OS [adjusted HR (95% CI): 1.67 (1.17–2.38), $p = 0.004$, Supplementary Table S3], as was ATB exposure in the 60 days [adjusted HR (95% CI): 1.47 (1.05–2.04), $p = 0.022$, Supplementary Table S4]. The proportion of responders was lower (1) in the ATB+30 group than in the ATB−30 group [10.5% *versus* 29.4% *versus* respectively; $p = 0.003$, Supplementary Figure S4(a)] and (2) in the ATB+60 group than in the ATB−60 group [9.0% *versus* 31.6%, respectively; $p < 0.001$, Supplementary Figure S4(b)]. The results of multivariate analyses showed that ATB exposure within 30 days was still associated with a worse tumor response [adjusted OR (95% CI): 3.11 (1.37–7.92), $p = 0.011$, Supplementary Table S5] as well as ATB exposure in the 60 days [adjusted OR (95% CI): 4.21 (1.95–10.05), $p < 0.001$, Supplementary Table S6].

In a sensitivity analysis that excluded patients who died within 4 weeks of ATB discontinuation ($n = 9$), OS was shorter in the ATB+ group than in the ATB− group [median (95% CI) OS time: 43.0 (36.1–56.9) *versus* 96.9 (64.1–152.4) weeks, respectively; crude HR (95% CI): 1.59 (1.19–2.12); $p = 0.001$ in a log-rank test; Supplementary Figure S5(a)]. There was no significant difference in OS between (1) patients having discontinued the ICI due to the infection and those not having discontinued the ICI [median (95% CI) OS time: 31.1 (23.7–NA) *versus* 39.9 (30.9–54.7) weeks, respectively; crude HR (95% CI): 1.06 (0.62–1.81), $p = 0.840$ in a log-rank test, Supplementary Figure S5(b)], (2) patients hospitalized for an infection and those not hospitalized [median (95% CI) OS time: 26.9 (22.3–46.9) *versus* 49.3 (36.1–68.9) weeks, respectively;

Table 3. Univariate and multivariate analysis of overall survival (Cox regression model).

	N (%)	Crude model		Adjusted model	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	372 (100)	1.00 (0.99–1.01)	0.556	1.01 (0.99–1.02)	0.307
Body mass index	372 (100)	0.95 (0.93–0.98)	<0.001	0.99 (0.95–1.02)	0.402
Sex					
Male	244 (65.6)	Reference		Reference	
Female	128 (34.4)	0.80 (0.60–1.06)	0.100	0.80 (0.57–1.10)	0.172
Smoking					
Never	104 (28.0)	Reference		Reference	
Current or past	268 (72.0)	1.65 (1.22–2.26)	0.001	0.98 (0.65–1.49)	0.936
Alcohol consumption					
No	247 (66.4)	Reference		Reference	
Yes	125 (33.6)	1.57 (1.20–2.06)	<0.001	1.20 (0.85–1.70)	0.303
History of cardiovascular disease					
Absence of cardiovascular disease	269 (72.3)	Reference		Reference	
Presence of cardiovascular disease	103 (27.7)	1.10 (0.82–1.46)	0.500	0.85 (0.62–1.16)	0.299
Type of cancer					
Lung	166 (44.6)	Reference		Reference	
Melanoma	110 (29.6)	0.45 (0.32–0.63)	<0.001	1.14 (0.61–2.15)	0.677
Renal and urothelial	27 (7.3)	0.76 (0.45–1.28)	0.306	0.89 (0.38–2.11)	0.789
Head and neck	48 (12.9)	1.05 (0.70–1.57)	0.824	0.69 (0.43–1.12)	0.129
Hodgkin lymphoma	5 (1.3)	0.45 (0.11–1.81)	0.260	0.47 (0.11–2.07)	0.318
Digestive	4 (1.1)	6.08 (2.19–16.85)	<0.001	4.03 (1.22–13.37)	0.023
Cutaneous squamous cell carcinoma	1 (0.3)	3.27 (0.45–23.58)	0.240	5.70 (0.69–49.80)	0.105
Adenocarcinoma of unknown primary	5 (1.3)	0.46 (0.11–1.86)	0.275	0.66 (0.16–2.79)	0.572
Squamous cell carcinoma of unknown primary	5 (1.3)	0.86 (0.27–2.70)	0.794	0.34 (0.10–1.16)	0.084
Porocarcinoma	1 (0.3)	2.11 (0.29–15.15)	0.459	2.80 (0.33–23.69)	0.343
Cancer duration	372 (100)	1.00 (0.99–1.00)	0.081	1.00 (0.99–1.00)	0.337
ECOG performance status					
0–1	295 (79.3)	Reference		Reference	
2–4	77 (20.7)	2.90 (2.15–3.90)	<0.001	2.73 (1.92–3.89)	<0.001

(Continued)

Table 3. (Continued)

	N (%)	Crude model		Adjusted model	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Metastatic cancer					
No	96 (25.8)	Reference		Reference	
Yes	276 (74.2)	0.74 (0.55–0.98)	0.040	0.89 (0.62–1.28)	0.538
Brain metastasis					
No	343 (92.2)	Reference		Reference	
Yes	29 (7.8)	1.13 (0.70–1.83)	0.600	1.25 (0.70–2.23)	0.453
Prior conventional chemotherapy					
No	153 (41.1)	Reference		Reference	
Yes	219 (58.9)	1.72 (1.30–2.28)	<0.001	1.53 (0.95–2.46)	0.081
Prior targeted chemotherapy					
No	306 (82.3)	Reference		Reference	
Yes	66 (17.7)	1.17 (0.83–1.63)	0.400	1.27 (0.78–2.07)	0.338
ICIs					
Nivolumab	217 (58.3)	Reference		Reference	
Pembrolizumab	130 (34.9)	0.58 (0.43–0.77)	<0.001	0.64 (0.44–0.94)	0.023
Ipilimumab	15 (4.0)	0.36 (0.17–0.78)	0.009	0.46 (0.18–1.15)	0.096
Nivolumab + ipilimumab	10 (2.7)	0.56 (0.21–1.50)	0.247	1.07 (0.36–3.24)	0.900
ATB use					
No	260 (69.9)	Reference		Reference	
Yes	112 (30.1)	1.75 (1.33–2.30)	<0.001	1.38 (1.00–1.90)	0.048
PPI use					
No	223 (59.9)	Reference		Reference	
Yes	149 (40.1)	0.84 (0.54–1.29)	0.150	0.80 (0.60–1.08)	0.148
Opioid use					
No	199 (53.5)	Reference		Reference	
Yes	173 (46.5)	1.82 (1.40–2.37)	<0.001	1.33 (0.99–1.79)	0.057
Metformin use					
No	355 (95.4)	Reference		Reference	
Yes	17 (4.6)	0.77 (0.40–1.51)	0.500	0.75 (0.36–1.58)	0.455

(Continued)

Table 3. (Continued)

	N (%)	Crude model		Adjusted model	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Levothyrox use					
No	332 (89.2)	Reference		Reference	
Yes	40 (10.8)	0.83 (0.54–1.29)	0.400	1.06 (0.65–1.72)	0.822
Oral corticosteroid use for an irAE					
No	351 (94.4)	Reference		Reference	
Yes	21 (5.6)	1.25 (0.53–1.71)	0.900	1.03 (0.55–1.94)	0.926
Oral corticosteroid use for another indication					
No	316 (84.9)	Reference		Reference	
Yes	56 (15.1)	1.35 (0.96–1.90)	0.087	1.33 (1.04–2.19)	0.046
Use of food supplements					
No	314 (84.4)	Reference		Reference	
Yes	58 (15.6)	1.58 (1.13–2.22)	0.007	1.00 (0.68–1.47)	0.990

ATB, antibiotic; AVK, anti-vitamin K; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; NSAID: nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor.

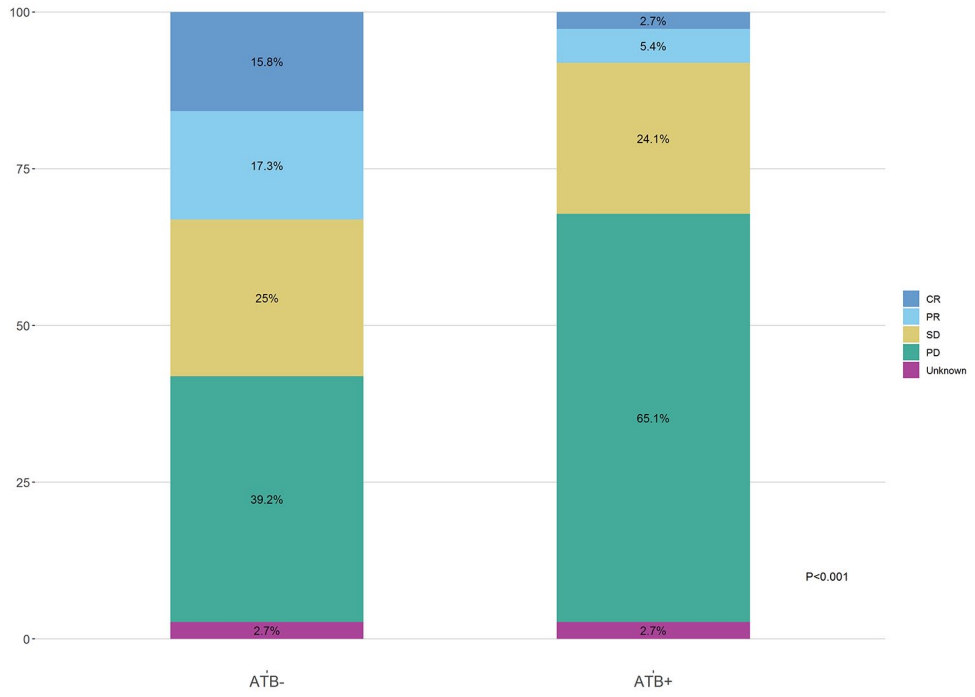

Figure 2. The best overall tumor response as a function of ATB use (the ATB– group *versus* the ATB+ group).
ATB, antibiotic; CR, complete response; PD, progression of disease; PR, partial response; SD, stable disease.

Table 4. Univariate and multivariate analysis (logistic regression) of the tumor response (comparison of nonresponders with responders, according to the RECIST 1.1 criteria).

	N (%)*	Crude model		Adjusted model	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age	362 (100)	1.00 (0.99–1.02)	0.640	1.01 (0.99–1.04)	0.307
Body mass index	362 (100)	0.94 (0.90–0.98)	0.003	1.00 (0.94–1.05)	0.867
Sex					
Male	237 (65.5)	Reference		Reference	
Female	125 (34.5)	0.73 (0.45–1.18)	0.193	0.68 (0.36–1.30)	0.241
Smoking					
Never	101 (27.9)	Reference		Reference	
Current or past	261 (72.1)	1.78 (1.07–2.93)	0.025	0.75 (0.35–1.62)	0.471
Alcohol consumption					
No	239 (66.0)	Reference		Reference	
Yes	123 (34.0)	1.51 (0.91–2.57)	0.115	0.87 (0.42–1.78)	0.693
History of cardiovascular disease					
Absence of cardiovascular disease	263 (72.7)	Reference		Reference	
Presence of cardiovascular disease	99 (27.3)	1.45 (0.85–2.57)	0.183	1.08 (0.55–2.16)	0.824
Type of cancer					
Lung	158 (43.6)	2.27 (1.39–3.80)	0.001	0.80 (0.19–2.73)	0.738
Melanoma	108 (29.3)	0.28 (0.17–0.46)	<0.001	0.77 (0.16–3.25)	0.730
Renal and urothelial	27 (7.5)	1.61 (0.64–4.94)	0.347	2.10 (0.29–14.73)	0.457
Head and neck	48 (13.3)	1.22 (0.62–2.63)	0.574	0.54 (0.10–2.40)	0.429
Cancer duration	362 (100)	0.99 (0.99–1.00)	0.045	0.99 (0.99–1.00)	0.055
ECOG performance status					
0–1	288 (79.6)	Reference		Reference	
2–4	74 (20.4)	3.57 (1.73–8.35)	0.001	2.71 (1.17–6.93)	0.026
Metastatic cancer					
No	95 (26.2)	Reference		Reference	
Yes	267 (73.8)	0.87 (0.50–1.47)	0.600	1.23 (0.59–2.56)	0.580
Brain metastasis					
No	335 (92.5)	Reference		Reference	
Yes	27 (7.5)	1.27 (0.52–3.54)	0.622	1.86 (0.55–6.98)	0.334
Prior conventional chemotherapy					
No	151 (41.7)	Reference		Reference	
Yes	211 (58.3)	2.60 (1.62–4.22)	<0.001	1.82 (0.76–4.35)	0.175

(Continued)

Table 4. (Continued)

	N (%)*	Crude model		Adjusted model	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Prior targeted chemotherapy					
No	296 (81.8)	Reference		Reference	
Yes	66 (18.2)	1.03 (0.57–1.94)	0.921	0.95 (0.39–2.40)	0.917
ICIs					
Nivolumab	209 (57.7)	Reference		Reference	
Pembrolizumab	128 (35.4)	0.48 (0.29–0.80)	0.004	0.73 (0.34–1.56)	0.408
Ipilimumab	15 (4.1)	0.49 (0.16–1.64)	0.212	1.23 (0.28–5.69)	0.780
Nivolumab + ipilimumab	10 (2.8)	0.16 (0.04–0.60)	0.007	0.24 (0.04–1.31)	0.107
ATB use					
No	253 (69.9)	Reference		Reference	
Yes	109 (30.1)	5.72 (2.90–12.67)	<0.001	6.06 (2.80–14.53)	<0.001
PPI use					
No	217 (59.4)	Reference		Reference	
Yes	145 (40.6)	1.87 (1.14–3.12)	0.015	1.57 (0.85–2.91)	0.150
Opioid use					
No	195 (53.9)	Reference		Reference	
Yes	167 (46.1)	2.60 (1.59–4.34)	<0.001	1.63 (0.88–3.06)	0.123
Metformin use					
No	345 (95.3)	Reference		Reference	
Yes	17 (4.7)	0.38 (0.14–1.04)	0.053	0.51 (0.14–1.76)	0.283
Levothyroxine use					
No	323 (89.2)	Reference		Reference	
Yes	39 (10.8)	0.89 (0.44–1.95)	0.768	1.44 (0.58–3.71)	0.434
Oral corticosteroid use for an irAE					
No	341 (94.2)	Reference		Reference	
Yes	21 (5.8)	0.88 (0.35–2.54)	0.803	1.57 (0.48–6.05)	0.480
Oral corticosteroid use for another indication					
No	311 (85.9)	Reference		Reference	
Yes	51 (14.1)	2.48 (1.14–6.21)	0.033	1.90 (1.03–5.45)	0.048
Use of food supplements					
No	304 (84.0)	Reference		Reference	
Yes	58 (16.0)	5.77 (2.28–19.45)	0.001	3.40 (1.14–13.09)	0.045

*10 patients were excluded from the analysis because of missing data for the best overall response.
ATB, antibiotic; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; OR, odds ratio; PPI, proton pump inhibitor.

crude HR (95% CI): 1.48 (0.95–2.30), log-rank $p = 0.081$, Supplementary Figure S5(c)], or (3) patients with a severe infection and those with a mild infection [median (95% CI) OS time: 26.9 (23.7–59.9) *versus* 45.6 (33.3–64.1) weeks, respectively; crude HR (95% CI): 1.31 (0.83–2.05), $p = 0.240$ in a log-rank test, Supplementary Figure S5(d)].

OS did not differ in patients treated with amoxicillin or amoxicillin/clavulanic acid ($n = 70$) *versus* patients treated with other ATBs [$n = 42$; median (95% CI) OS time: 40.1 (27.3–56.9) *versus* 37.6 (26.6–64.1) weeks, respectively; crude HR (95% CI): 0.91 (0.58–1.42), $p = 0.700$ in a log-rank test]. Furthermore, OS was significantly lower in patients treated with amoxicillin or amoxicillin/clavulanic acid than in patients in the ATB− group [median (95% CI) OS time: 40.1 (27.3–56.9) *versus* 96.9 (64.1–152.4) weeks, respectively; crude HR (95% CI): 1.69 (1.22–2.34), $p = 0.001$ in a log-rank test]. Lastly, OS did not differ in patients treated with amoxicillin ($n = 9$) *versus* those treated with amoxicillin/clavulanic acid [$n = 61$; median (95% CI) OS time: 31.0 (21.0–NA) *versus* 42.7 (27.3–NA) weeks, respectively; crude HR (95% CI): 0.68 (0.32–1.46), $p = 0.330$ in a log-rank test, Supplementary Figure S6].

Patients with melanoma. Overall, 16 of the 110 patients in the melanoma subgroup (14.5%) received ATBs. The baseline characteristics were similar in the ATB− and ATB+ groups, except that the proportion of PPI users was lower in the ATB+ group than in the ATB− group (12.5% *versus* 39.4%, respectively; $p = 0.048$, Supplementary, Table S7). OS was shorter in the melanoma ATB+ group than in the melanoma ATB− group [median (95% CI) OS time: 68.9 (21.3–NA) *versus* 291.0 (128.9–NA) weeks, respectively; crude HR (95% CI): 2.27 (1.09–4.71), log-rank $p = 0.024$, Supplementary Figure S7]. The Cox proportional hazards model confirmed this result [adjusted HR (95% CI): 2.60 (1.06–6.39), $p = 0.037$]. Furthermore, the use or oral corticosteroids for an indication other than an irAE was associated with significantly poorer OS [adjusted HR (95% CI): 4.84 (1.86–12.64), $p < 0.001$], whereas the use of corticosteroids for an irAE was not [adjusted HR (95% CI): 3.33 (0.61–18.30), $p = 0.167$, Supplementary, Table S8].

The proportion of responders was higher in the melanoma ATB− group than in the melanoma ATB+ group (46.8% *versus* 25.0%) but this difference was not significant ($p = 0.116$, Supplementary Figure S8). After adjustment for confounding variables, the logistic regression analysis showed a significant association between ATB use and a worse tumor response [adjusted OR (95% CI): 4.41 (1.04–22.80), $p = 0.045$]. Furthermore, the use of corticosteroids for an indication other than an irAE was associated with a worse tumor response [adjusted OR (95% CI): 4.97 (1.05–65.83), $p = 0.039$] whereas the use of corticosteroids for an irAE was not [adjusted HR (95% CI): 1.96 (0.15–31.10), $p = 0.609$, Supplementary Table S9].

Other drugs. OS did not differ significantly when comparing patients having received NSAIDs, PPIs, statins, AVK anticoagulants, levothyroxine, cholecalciferol, phloroglucinol, metformin or antiarrhythmics with patients not having received these drugs (Supplementary Figure S9). The same was true for the tumor response, except for patients treated with PPIs (response rate: 18.8% *versus* 30.1% in the other patients; $p = 0.036$) or metformin (response rate: 47.1% *versus* 24.5% in the other patients; $p = 0.020$; Supplementary Figure S10). Since only one patient received psychotropic medication (escitalopram), we did not analyze that drug class.

In patients treated with opioids (the Opioids+ group), the median (95% CI) OS time was significantly shorter than in patient who were not treated with opioids [the Opioids− group; 36.6 (26.6–48.1) *versus* 126.4 (77.7–168.4) weeks, respectively; crude HR (95% CI): 1.82 (1.40–2.37), $p < 0.001$, Supplementary Figure S9(d)]. Moreover, the response rate in the Opioids+ group was lower than in the Opioids− group [16.2 *versus* 33.7, respectively; $p < 0.001$, Supplementary Figure S10(d)]. In bivariate analyses, patients in the Opioids+ group had a worse prognosis than patients in the Opioids− group: the mean ± SD BMI was lower (23.5 ± 5.0 *versus* 25.9 ± 5.6, respectively; $p < 0.001$), the prevalence of smoking was higher (82.1% *versus* 63.3%, respectively; $p < 0.001$), the prevalence of alcohol consumption was higher (39.3% *versus* 28.6%, respectively; $p = 0.039$) and the proportion of patients with a poor ECOG performance status was higher (31.2% *versus* 11.4%, $p < 0.001$, respectively; Supplementary, Table S10). This trend was no longer significant after adjustment for confounding factors [adjusted HR (95% CI): 1.33 (0.99–1.79), $p = 0.057$, Table 3].

The characteristics of patients taking NSAIDs, PPIs, statin, AVK, levothyroxine, cholecalciferol, antiarrhythmic, metformin, phloroglucinol or corticosteroids are summarized in Supplementary Tables S10–S20.

Discussion

Our present results showed that in a population of patients receiving ICIs (regardless of the indication), treatment with ATBs was associated with poorer OS [adjusted HR (95% CI): 1.38 (1.00–1.90); $p = 0.048$] and a worse tumor response [adjusted OR (95% CI): 6.06 (2.80–14.53), $p < 0.001$]. These findings are in line with some of the literature data,[10,16,17,19,22] with a similar effect size (the adjusted HR) for OS. However, other studies have not found a negative association between ATB use and OS or the tumor response.[15,20,21] There are several possible explanations for this disparity in the literature data. Firstly, it is difficult to set an optimal time cut-off when considering whether or not ATB use influences the effectiveness of ICIs. For example, the findings of Derosa *et al.*[22] varied as a function of the cut-off: ATBs were associated with poorer OS when the treatment was initiated 30 days before ICI initiation but not when the cut-off considered was 60 days. This prompted us to take account of the time needed for the gut microbiota to recover after ATB discontinuation (according to the literature) for each molecule.[13] However, the results of our sensitivity analyses showed poorer OS and a worse tumor response regardless of the cut-off (ATB exposure in the 30 or 60 days preceding or following ICI initiation). Secondly, the nature of the dysbiosis differs from one class of ATBs to another.[13] For instance, vancomycin might boost the antitumor effects of CTLA-4 blockade, presumably by inducing an increase in the proportion of Bacteroidales.[14] Again, we took this parameter in account by consulting the literature data.[13] Thirdly, the previously published studies did not consider indication bias (i.e. with death being linked to the infection that prompted ATB use). In the present study, we minimized this bias by excluding patients who died or were lost to follow-up within 4 weeks of ATB discontinuation (considering that censorship could be linked to death); after this adjustment, the OS time was still shorter in the ATB+ group than in the ATB− group [crude HR (95% CI): 1.59 (1.19–2.12); $p = 0.001$]. Within the ATB+ group, there was no difference in OS between patients with severe infections [i.e. those requiring hospitalization (crude HR (95% CI): 1.48 (0.95–2.30), $p = 0.081$)] or who met the international criteria for sepsis[39] [crude HR (95% CI): 1.31 (0.83–2.05), $p = 0.240$] and the other patients, or between patients who discontinued ICIs as a result of the infection and the other patients [crude HR (95% CI): 1.06 (0.62–1.81), $p = 0.840$]. Hence, these factors did not explain why OS was worse in the ATB+ group. Furthermore, recent *post hoc* analyses of randomized trials showed a negative impact of ATB use in patients treated with the anti-PD-L1 atezolizumab, relative to those in the control arm (conventional chemotherapy), reinforcing the validity of our results.[23,24]

The vast majority of the previously cited studies focused on NSCLC, renal cell carcinoma or UC. Very few published studies have examined the impact of ATB use on OS and the tumor response in melanoma patients treated with ICIs. There were 10 patients with melanoma in Iglesias-Santamaría's study[21] and 179 in the study of Tinsley *et al.*[19] but neither of the studies specifically studied the association between ATB use and OS. In the present study, OS was lower in the ATB+ group than in the ATB− group when considering the 110 patients with melanoma [adjusted HR (95% CI): 2.60 (1.06–6.39), $p = 0.037$], and ATB use was associated with a worse tumor response in this population [adjusted OR (95% CI): 4.41 (1.04–22.80), $p = 0.045$], despite the fact that few ($n = 16$, 14.5%) had received ATBs. This finding emphasizes the significance of our results.

We also focused on patients receiving the β-lactamase inhibitor amoxicillin or amoxicillin/clavulanic acid, since (1) these drugs might increase the abundance of the Bacteroidales and *Bifidobacterium* species in the gut microbiota,[13] which appear to boost the effectiveness of ICIs,[10,14,37,38] and (2) this class of ATB is widely prescribed. However, in our population, the OS (95% CI) time was significantly shorter in the amoxicillin or amoxicillin/clavulanic acid group than in the ATB− group [40.1 (27.3–56.9) *versus* 96.9 (64.1–152.4) weeks, respectively; crude HR (95% CI): 1.69 (1.22–2.34), $p = 0.001$ in a log-rank test], showing that the use of these drugs is also associated with poorer clinical outcomes.

In the present study, exposure to other known dysbiosis-inducing drugs (except for opioids) was not associated with a difference in OS, perhaps

because of a low impact on the gut microbiota. However, it has been demonstrated that these drug classes induce dysbiosis. The use of PPIs is associated with a greater abundance of *Bifidobacterium* species,[44] which might boost the effectiveness of ICIs, but also with a decrease in the alpha diversity of the gut microbiota. Alpha diversity appears to be associated with higher response rates in ICI-treated patients with melanoma.[45,46] These changes in the gut microbiota result from a decrease in stomach acidity and the compounds direct effects.[28,45–46] Retrospective studies have given conflicting results; some have shown poorer clinical outcomes in ICI-treated patients exposed to PPIs,[47] while others did not show a significant association[15,21] and yet others showed better clinical outcomes in this population.[48,49] In the present study, we failed to show an association between PPI use and clinical outcomes (OS and the tumor response) in multivariate analyses. However, recent *post hoc* analyses of randomized trials, with larger cohorts and without the inherent biases of retrospective studies, showed a negative impact of PPI use on OS and progression-free survival in patients treated with ICIs compared with patients treated with conventional chemotherapy.[24,50] There are several possible explanations for these conflicting results. Firstly, the small sample size of retrospective studies, including ours, leads to a low statistical power and might prevent the identification of associations. Secondly, the heterogeneity of the population in retrospective studies (in terms of the type of cancer, the number of previous lines of chemotherapy, the type of ICI (anti-PD-L1 and anti-CTLA-4 agents) and the baseline characteristics) might create issues that might not be addressed in the multivariate analyses and might increase residual confounding bias. Indeed, the randomized trials performed by Hopkins *et al.*[50] and by Chalabi *et al.*[24] focused on patients with UC and NSCLC, respectively, and all the patients in the ICI arm were treated with the anti-PD-L1 atelozumab. Moreover, all these patients were treatment-naïve or had been treated with only one or two cytotoxic chemotherapy regimens before inclusion in the trials, and none had received targeted chemotherapy. In contrast, some of patients in our cohort had received many lines of treatment, including targeted chemotherapy. Indeed, the PPIs' dysbiosis effect on ICI may be more pronounced during early lines of treatment because late-stage tumor cells can evolve novel strategies to escape immunosurveillance.[51]

Opioid use might affect the composition of the gut microbiota by activating opioid receptors and thus reducing intestinal motility.[34] Moreover, tramadol shows bactericidal activity *in vitro*,[52] and a clinical study has described opioid-induced dysbiosis.[35] In the present study, however, opioid use was associated with poorer OS in a univariate analysis but not in a multivariate analysis. In our population, patients treated with opioids had a worse prognosis (particularly with regard to ECOG performance status) than those who were not. However, the use of opioids in patients with a worse prognosis might explain these findings. In Iglesias-Santamaría's study,[21] opioid use was associated with poorer OS in a multivariate analysis [adjusted HR (95% CI): 3.63 (1.92–6.85); $p < 0.001$]. However, this difference might be due to the fact that (in contrast to the present study) Iglesias-Santamaría did not included ECOG performance status in the Cox model.

The present study also considered statins, NSAIDs, AVK anticoagulants, cholecalciferol, phloroglucinol, and antiarrhythmics, all of which are known to induce dysbiosis.[26,28,31–33,46] We did not observe an association between the use of any of these drugs and OS. One can hypothesize that these drugs have less of an effect on the composition of the gut microbiota than ATBs do. Moreover, the low number of patients taking NSAIDs, AVK anticoagulants, phloroglucinol, metformin, and antiarrhythmics might have prevented us from evidencing significant associations. With regard to metformin and statins (which appear to have a protective role on gut microbiota[26,27]), we failed to show a positive association with OS or the tumor response (except for metformin for the tumor response in bivariate analysis), as was also the case in the study of Failing *et al.*[49] This absence of an association could be related to (1) the low number of patients treated with metformin in our cohort, as mentioned above, and (2) the indications of these drugs themselves (i.e. comorbidity factors).

We found that corticosteroid use for an indication other than an irAE was associated with poorer OS and a worse tumor response, whereas corticosteroid use for an irAE was not. The fact that patients who experience irAEs survive for longer than patients without irAEs might explain these results.[53–55] This finding is in line with studies showing that (1) the early use of corticosteroids

(which is not related to irAEs) is harmful upon ICI initiation [40–42] and (2) the use of corticosteroids to treat irAEs during immunotherapy does not affect OS.[56–58] Therefore, the fact that corticosteroids are not associated with poorer OS or a worse tumor response in the setting of an irAE argues that it is the indication for steroids and not the steroids themselves that affect OS. Further studies with a comparative arm are needed to explore this point.

Our study had several limitations. Firstly, the single-center, retrospective design resulted in inherent bias, including the heterogeneity of the study population. Secondly, data on several confounding factors for comorbidity in the general population (such as congestive heart failure, dementia, chronic lung disease, chronic kidney failure, connective disease, peptic ulcer, hemiplegia, cirrhosis, and HIV infection, as described in the Charlson index)[59] are lacking. Furthermore, specific prognostic factors for cancer were missing, such as PD-L1 tumor proportion score, the tumor mutational burden, the Breslow thickness or the Clark index in melanoma population. Thirdly, in the absence of a comparator arm, it is difficult to affirm that the use of any drug class has an impact on treatment effectiveness, even though we sought to minimize indication bias much as possible by performing sensitivity analyses. Fourthly, the study probably lacked statistical power for some of the analyses, especially with regards to NSAIDs, metformin, AVK anticoagulants, phloroglucinol, and antiarrhythmics. No power thresholds were predefined. Fifthly, we did not study the gut microbiota *per se* and so could not directly confirm the induction of dysbiosis in our patients. Lastly, a number of factors known to influence the gut microbiota (such as diet, country of origin, and breast feeding) were not documented.

Conclusion

The use of ATBs (depending on their specific effects on the gut microbiota and the time needed for recovery of the latter following discontinuation) is associated with poorer OS and a lower tumor response rate in patients treated with ICIs (especially in patients with melanoma), regardless of the severity of infection. ATBs should be used with caution in clinical practice, and the prescribing physician should always take account of the risk/benefit ratio.

Author contribution statement

Louis Gaucher contributed to the conception/design of the work, acquisition and interpretation of data for the work, and drafting the manuscript. He approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Leslie Adda contributed to acquisition and interpretation of data for the work. She approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Alice Séjourné contributed to acquisition and interpretation of data for the work, and drafting the manuscript. She approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Camille Joachim revised the work critically for important intellectual content, approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Claire Poulet revised the work critically for important intellectual content, approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Kamel Masmoudi revised the work critically for important intellectual content, approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Aline Houessinon revised the work critically for important intellectual content, approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Youssef Bennis contributed to the conception/design of the work and interpretation of data for the work, and drafting the manuscript. He approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Benjamin Batteux contributed to the conception/design of the work, acquisition, analysis, and interpretation of data for the work, and drafting the manuscript. He approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest statement

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Supplemental material

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References

1. Wolchok JD and Saenger Y. The mechanism of anti-CTLA-4 activity and the negative regulation of T-cell activation. *Oncologist* 2008; 13(Suppl. 4): 2–9.
2. Alsaab HO, Sau S, Alzhrani R, *et al.* PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Front Pharmacol* 2017; 8: 561.
3. McDermott D, Lebbé C, Hodi FS, *et al.* Durable benefit and the potential for long-term survival with immunotherapy in advanced melanoma. *Cancer Treat Rev* 2014; 40: 1056–1064.
4. Rodríguez-Cerdeira C, Carnero Gregorio M, López-Barcenas A, *et al.* Advances in immunotherapy for melanoma: a comprehensive review. *Mediators Inflamm* 2017; 2017: 3264217.
5. Ruiz-Patiño A, Arrieta O, Cardona AF, *et al.* Immunotherapy at any line of treatment improves survival in patients with advanced metastatic non-small cell lung cancer (NSCLC) compared with chemotherapy (Quijote-CLICaP). *Thorac Cancer* 2020; 11: 353–361.
6. Haslam A and Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. *JAMA Netw Open* 2019; 2: e192535.
7. Chen DS and Mellman I. Elements of cancer immunity and the cancer–immune set point. *Nature* 2017; 541: 321–330.
8. Ricciuti B, Dahlberg SE, Adeni A, *et al.* Immune checkpoint inhibitor outcomes for patients with non-small-cell lung cancer receiving baseline corticosteroids for palliative versus nonpalliative indications. *J Clin Oncol* 2019; 37: 1927–1934.
9. Gaboriau-Routhiau V and Cerf-Bensussan N. [Gut microbiota and development of the immune system]. *Med Sci (Paris)* 2016; 32: 961–967.
10. Routy B, Le Chatelier E, Derosa L, *et al.* Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; 359: 91–97.
11. Humphries A and Daud A. The gut microbiota and immune checkpoint inhibitors. *Hum Vaccin Immunother* 2018; 14: 2178–2182.
12. Inamura K. Roles of microbiota in response to cancer immunotherapy. *Semin Cancer Biol* 2020; 65: 164–175.
13. Zimmermann P and Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. *J Infect* 2019; 79: 471–489.

14. Vétizou M, Pitt JM, Daillère R, *et al*. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015; 350: 1079–1084.

15. Hakozaki T, Okuma Y, Omori M, *et al*. Impact of prior antibiotic use on the efficacy of nivolumab for non-small cell lung cancer. *Oncol Lett* 2019; 17: 2946–2952.

16. Huemer F, Rinnerthaler G, Westphal T, *et al*. Impact of antibiotic treatment on immune-checkpoint blockade efficacy in advanced non-squamous non-small cell lung cancer. *Oncotarget* 2018; 9: 16512–16520.

17. Ueda K, Yonekura S, Ogasawara N, *et al*. The impact of antibiotics on prognosis of metastatic renal cell carcinoma in Japanese patients treated with immune checkpoint inhibitors. *Anticancer Res* 2019; 39: 6265–6271.

18. Pinato DJ, Gramenitskaya D, Altmann DM, *et al*. Antibiotic therapy and outcome from immune-checkpoint inhibitors. *J Immunother Cancer* 2019; 7: 287.

19. Tinsley N, Zhou C, Tan G, *et al*. Cumulative antibiotic use significantly decreases efficacy of checkpoint inhibitors in patients with advanced cancer. *Oncologist* 2020; 25: 55–63.

20. Kaderbhai C, Richard C, Fumet JD, *et al*. Antibiotic use does not appear to influence response to nivolumab. *Anticancer Res* 2017; 37: 3195–3200.

21. Iglesias-Santamaría A. Impact of antibiotic use and other concomitant medications on the efficacy of immune checkpoint inhibitors in patients with advanced cancer. *Clin Transl Oncol*. Epub ahead of print 9 January 2020. DOI: 10.1007/s12094-019-02282-w.

22. Derosa L, Hellmann MD, Spaziano M, *et al*. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann Oncol* 2018; 29: 1437–1444.

23. Hopkins AM, Kichenadasse G, Karapetis CS, *et al*. Concomitant antibiotic use and survival in urothelial carcinoma treated with atezolizumab. *Eur Urol* 2020; 78: 540–543.

24. Chalabi M, Cardona A, Nagarkar DR, *et al*. Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled post hoc analyses of the OAK and POPLAR trials. *Ann Oncol* 2020; 31: 525–531.

25. Fakhoury HMA, Kvietys PR, AlKattan W, *et al*. Vitamin D and intestinal homeostasis: barrier, microbiota, and immune modulation. *J Steroid Biochem Mol Biol* 2020; 200: 105663.

26. Pollak M. The effects of metformin on gut microbiota and the immune system as research frontiers. *Diabetologia* 2017; 60: 1662–1667.

27. Dias AM, Cordeiro G, Estevinho MM, *et al*. Gut bacterial microbiome composition and statin intake-a systematic review. *Pharmacol Res Perspect* 2020; 8: e00601.

28. Maier L, Pruteanu M, Kuhn M, *et al*. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018; 555: 623–628.

29. Bruno G, Zaccari P, Rocco G, *et al*. Proton pump inhibitors and dysbiosis: current knowledge and aspects to be clarified. *World J Gastroenterol* 2019; 25: 2706–2719.

30. Wu H, Esteve E, Tremaroli V, *et al*. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017; 23: 850–858.

31. Caparrós-Martín JA, Lareu RR, Ramsay JP, *et al*. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. *Microbiome* 2017; 5: 95.

32. Charoenngam N, Shirvani A, Kalajian TA, *et al*. The effect of various doses of oral vitamin D3 supplementation on gut microbiota in healthy adults: a randomized, double-blinded, dose-response study. *Anticancer Res* 2020; 40: 551–556.

33. Rogers MAM and Aronoff DM. The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. *Clin Microbiol Infect* 2016; 22: 178. e1–178.e9.

34. Cussotto S, Clarke G, Dinan TG, *et al*. Psychotropics and the microbiome: a chamber of secrets. . .. *Psychopharmacology (Berl)* 2019; 236: 1411–1432.

35. Acharya C, Betrapally NS, Gillevet PM, *et al*. Chronic opioid use is associated with altered gut microbiota and predicts readmissions in patients with cirrhosis. *Aliment Pharmacol Ther* 2017; 45: 319–331.

36. Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228–247.

37. Sivan A, Corrales L, Hubert N, *et al*. Commensal bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015; 350: 1084–1089.

38. Salek Farrokhi A, Darabi N, Yousefi B, *et al.* Is it true that gut microbiota is considered as panacea in cancer therapy? *J Cell Physiol*. Epub ahead of print 20 February 2019. DOI: 10.1002/jcp.28333.
39. Levy MM, Fink MP, Marshall JC, *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Crit Care Med* 2003; 31: 1250–1256.
40. Arbour KC, Mezquita L, Long N, *et al.* Impact of baseline steroids on efficacy of programmed cell death-1 and programmed death-ligand 1 blockade in patients with non-small-cell lung cancer. *J Clin Oncol* 2018; 36: 2872–2878.
41. Scott SC and Pennell NA. Early use of systemic corticosteroids in patients with advanced NSCLC treated with nivolumab. *J Thorac Oncol* 2018; 13: 1771–1775.
42. Chasset F, Pages C, Biard L, *et al.* Single-center study under a French Temporary Authorization for Use (TAU) protocol for ipilimumab in metastatic melanoma: negative impact of baseline corticosteroids. *Eur J Dermatol* 2015; 25: 36–44.
43. Bartlett EK and Karakousis GC. Current staging and prognostic factors in melanoma. *Surg Oncol Clin N Am* 2015; 24: 215–227.
44. Jackson MA, Goodrich JK, Maxan M-E, *et al.* Proton pump inhibitors alter the composition of the gut microbiota. *Gut* 2016; 65: 749–756.
45. Imhann F, Bonder MJ, Vich Vila A, *et al.* Proton pump inhibitors affect the gut microbiome. *Gut* 2016; 65: 740–748.
46. Le Bastard Q, Al-Ghalith GA, Grégoire M, *et al.* Systematic review: human gut dysbiosis induced by non-antibiotic prescription medications. *Aliment Pharmacol Ther* 2018; 47: 332–345.
47. Homicsko K, Richtig G, Tuchmann F, *et al.* Proton pump inhibitors negatively impact survival of PD-1 inhibitor based therapies in metastatic melanoma patients. *Ann Oncol* 2018; 29(Suppl. 10): x40.
48. Afzal MZ and Shirai K. What impact do the proton pump inhibitors have on the efficacy of immune check point inhibitors in metastatic malignant melanoma? *J Clin Oncol* 2019; 37(Suppl. 15): e21040.
49. Failing JJ, Finnes HD, Kottschade LA, *et al.* Effects of commonly used chronic medications on the outcomes of ipilimumab therapy in patients with metastatic melanoma. *Melanoma Res* 2016; 26: 609–615.
50. Hopkins AM, Kichenadasse G, Karapetis CS, *et al.* Concomitant proton pump inhibitor use and survival in urothelial carcinoma treated with atezolizumab. *Clin Cancer Res* 2020; 26: 5487–5493.
51. Vivarelli S, Salemi R, Candido S, *et al.* Gut microbiota and cancer: from pathogenesis to therapy. *Cancers (Basel)*. Epub ahead of print 3 January 2019. DOI: 10.3390/cancers11010038.
52. Tamanai-Shacoori Z, Shacoori V, Jolivet-Gougeon A, *et al.* The antibacterial activity of tramadol against bacteria associated with infectious complications after local or regional anesthesia. *Anesth Analg* 2007; 105: 524–527.
53. Freeman-Keller M, Kim Y, Cronin H, *et al.* Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. *Clin Cancer Res* 2016; 22: 886–894.
54. Teulings H-E, Limpens J, Jansen SN, *et al.* Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J Clin Oncol* 2015; 33: 773–781.
55. Okada N, Kawazoe H, Takechi K, *et al.* Association between immune-related adverse events and clinical efficacy in patients with melanoma treated with nivolumab: a multicenter retrospective study. *Clin Ther* 2019; 41: 59–67.
56. Horvat TZ, Adel NG, Dang TO, *et al.* Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at memorial Sloan Kettering cancer center. *J Clin Oncol* 2015; 33: 3193–3198.
57. Kapoor A, Noronha V, Patil VM, *et al.* Concomitant use of corticosteroids and immune checkpoint inhibitors in patients with solid neoplasms: a real-world experience from a tertiary cancer center. *Cancer Res Stat Treat* 2019; 2: 204–208.
58. Weber JS, Larkin JMG, Schadendorf D, *et al.* Management of gastrointestinal (GI) toxicity associated with nivolumab (NIVO) plus ipilimumab (IPI) or IPI alone in phase II and III trials in advanced melanoma (MEL). *J Clin Oncol* 2017; 35(Suppl. 15): 9523.
59. Charlson ME, Pompei P, Ales KL, *et al.* A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–383.