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No detrimental association between antibiotic use and immune checkpoint inhibitor therapy: an observational cohort study comparing patients with ICI-treated and TKI-treated melanoma and NSCLC

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ABSTRACT

Background The role of antibiotics in malignancies treated with immune checkpoint inhibitors (ICI) remains unclear. Several studies suggested a detrimental impact of antibiotic use on the response to ICI, but were susceptible to confounding by indication. Our objective was therefore to assess whether the relationship between antibiotic use and ICI response is causative or merely associative.

Methods A large, single-center observational cohort study was performed with individuals treated for either non-small cell lung carcinoma (NSCLC) or metastatic melanoma. An effect modification approach was used, aiming to estimate the association between antibiotic use and overall survival (OS) and compare these estimates between individuals receiving first-line ICI treatment versus those receiving first-line tyrosine kinase inhibitors (TKIs). Exposure of interest was antibiotic use within 30 days before the start of anticancer treatment. HRs for OS were estimated for antibiotics versus no antibiotics in each cohort using multivariable propensity adjusted analysis. The "true antibiotic effect" within the ICI versus TKI cohort was modeled using an interaction term.

Results A total of 4534 patients were included, of which 1908 in the ICI cohort and 817 in the TKI cohort. Approximately 10% of patients in each cohort used antibiotics within 30 days before the start of anticancer treatment. Our results demonstrate a lack of synergistic interaction between current antibiotic use and ICI therapy in relation to OS: although antibiotic use was significantly associated with OS decline in the ICI cohort (HR=1.26 (95% CI 1.04 to 1.51)), a similar magnitude in OS decline was found within the TKI cohort (HR=1.24 (95% CI 0.95 to 1.62)). This was reflected by the synergy index (HR=0.96 (95% CI 0.70 to 1.31)), which implied no synergistic interaction between current antibiotic use and ICI.

Conclusion This study strongly suggests that there is no causal detrimental association between antibiotic use and ICI therapy outcome when looking at OS in individuals with malignant melanoma or NSCLC. The frequently observed inverse association between antibiotics and ICI response in previous studies is most likely driven by confounding by indication, which was confirmed by the findings in our reference TKI cohort.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Many retrospective cohort studies have shown a detrimental effect of antibiotics on survival among patients with immune checkpoint inhibitors (ICI)-treated cancer. Because the majority of these studies only include ICI-treated patients, the results of these studies are susceptible to confounding by indication.

WHAT THIS STUDY ADDS

⇒ This study deals with confounding by indication by including a cohort of tyrosine kinase inhibitors-treated patients as controls. The results of this study strongly suggest there is no causal relationship between use of antibiotics and ICI therapy outcome among patients with melanoma and non-small cell lung carcinoma.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ While clinicians should remain judicious in the use of antibiotics in general, concomitant use of ICI therapy does not seem to form a contraindication and continued use of antibiotics is warranted in justified underlying diseases.

BACKGROUND

Immune checkpoint inhibitors (ICI) are a more and more commonly used therapy for various oncologic indications, including melanoma and non-small cell lung cancer (NSCLC). These monoclonal antibodies targeting immune checkpoints cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death ligand 1

(PD-(L)1) have shown major clinical results, improving survival with better tolerated toxicities compared with chemotherapy. Only a subset of patients benefits from ICI's, however, while other patient groups show no response at all.[1–3] A host of other immune checkpoint targets are currently under investigation, promising to widen the scope of ICI therapy only further.[4] One of the key goals of ICI research is to find predictors of response to ICI and ways to improve response among patients.[5]

The potential role of the microbiome on efficacy and toxicity of ICI's is of increasing interest, due to the interplay between the microbiome and the immune system.[6] The composition of the microbiome (made up of commensal bacteria, viruses, yeast, fungi, archaea and protozoa) remains relatively stable throughout life, but transient dysbiosis can have a major impact on immune and inflammatory responses.[7] Antibiotic therapy can cause changes in the microbiome composition and function,[8] leading to the hypothesis that use of antibiotics before and/or during ICI therapy may impact survival rates. Although the body of evidence for interactions between the microbiome and immune response is large and ever growing, the precise mechanisms responsible for these interactions are still little understood. Whether a possible interplay between antibiotic use, microbiome dysbiosis and response to ICI therapy is of any clinical relevance remains unsure.

So far, clinical evidence on the association between antibiotic use and ICI response remains conflicting.[9 10] Most observational studies found a detrimental effect of antibiotics on overall survival (OS) and progression-free survival (PFS) following ICI therapy, but are susceptible to severe confounding by indication. It remains unclear whether antibiotic use truly causally interferes with ICI efficacy or whether antibiotic use is simply a marker of poor prognosis. Indeed, in other clinical, non-cancer-related fields, observational studies on antibiotic use typically report an increased rate of death in general, whereas randomized controlled trials do not,[11] underlining the concerns for confounding by indication. To overcome this limitation, Cortellini *et al* studied the effect of antibiotics on treatment response not only in patients with ICI-treated NSCLC, but also in a comparator arm of patients with chemotherapy-treated NSCLC.[12] Their results showed a detrimental effect of antibiotics on survival in the ICI treated cohort, but not in the chemotherapy treated cohort, supporting a potential causative role for antibiotics in ICI response. However, the selection of their chemotherapy reference group might have introduced sources of potential bias: antibiotics might enhance OS in chemotherapy patients preferentially (by preventing severe infections due to leukopenia) and calendar time differed substantially between their chemotherapy and ICI cohort. In addition, they were not able to explore additional time trends, which may help in evaluating plausibility of causal interference.[13]

To overcome the previously mentioned limitations, a large observational cohort study was performed with individuals treated for either NSCLC or metastatic melanoma. An effect modification approach was used, aiming to estimate the association between antibiotic use and OS and compare these estimates between individuals on ICI treatment versus those treated with tyrosine kinase inhibitors (TKIs).

METHODS

Design and study population

This observational study was carried out at the Netherlands Cancer Institute (NKI), a high-volume, specialized cancer hospital. Between January 1 2011 and December 31 2018, all individuals with a first diagnosis of malignant melanoma or NSCLC were selected and stratified into one of the following cohorts based on their choice of first-line treatment:

- ICI cohort: All patients receiving first-line PD-(L)1 or CTLA-4 targeted monoclonal antibody (ATC (anatomical therapeutic chemical classification system) codes starting with L01FF and ATC code L01F×04).
- TKI cohort: All patients receiving a first-line TKI, specifically anti-estimated glomerular filtration rate (eGFR), anti-ALK (anaplastic lymphoma kinase) or anti-BRAF (B-rapidly accelerated sarcoma) (ATC codes starting with L01EB, L01ED and L01EC, respectively).
- Chemotherapy cohort: All patients receiving first-line chemotherapy (ATC-codes starting with L01A, L01B, L01C and L01D).
- Chemoimmunotherapy cohort: All patients receiving first-line combined chemoimmunotherapy (ATC codes: see ICI and chemotherapy cohorts above).

Individuals with a different choice of first-line treatment were excluded. Data were collected from the NKI electronic patient records. Collected data included age, gender, underlying malignancy, type of cancer treatment, date of commencement of cancer treatment, organ function at baseline (eGFR, alanine aminotransferase (ALAT) blood levels, thyroid-stimulating hormone (TSH) blood levels), comedication use before and during cancer treatment, dates of commencement and date of end of comedication use. All patients were followed until death or end of data collection (December 31 2018), whichever came first. The study was performed in accordance with the ethical standards of the Helsinki declaration. All patients provided informed consent.

Exposure, outcomes and confounders

For each cohort, exposure to systemic antibiotic use (ATC codes starting with J01, J04, A07AA and A02BD) prior to the start of cancer treatment was assessed. Timing of antibiotic use was grouped according to the time window of antibiotic use respective to the start of cancer treatment (≤30 days (current users), 31–90 days, 91–365 days, >365 days before the start of cancer treatment). Patients without any recorded evidence for antibiotic use prior to the start of cancer treatment were considered non-users.

Exposure to oral and topical antibiotic use during cancer treatment was also assessed for each cohort.

Primary outcome was OS, defined as the time from the start of cancer treatment to death of any cause. Secondary outcome was a composite endpoint of therapy switch and death. Therapy switch was defined as the start of second-line cancer treatment for the same underlying malignant disease.

We reviewed literature to assess potential confounders. These include age, gender, primary tumor type (melanoma or NSCLC), tumor stage and presence of metastases at baseline, concomitant current use (within 3 months of cancer treatment commencement) of systemic corticosteroids, opioids or RAAS-inhibitors (renin-angiotensin-aldosteron system), lactate dehydrogenase blood level and neutrophil lymphocyte ratio at baseline eGFR, ALAT, TSH, C reactive protein, white blood cell count. All confounders, including age and clinical laboratory findings, were treated as categorical variables, based on distribution and biological sense. Missing data were handled using multiple imputations.

Analysis

Baseline patient characteristics were described according to anticancer treatment type (ICI-cohort, TKI-cohort, chemoimmunotherapy cohort and chemotherapy cohort) and antibiotics status (current antibiotic users and non-antibiotic users) within each cohort. HRs and 95% CIs for OS were estimated for antibiotics versus no antibiotics in each cohort using crude (age and gender adjusted) and propensity adjusted cox regression models. Propensity scores were calculated using logistic regression and all potential confounders were modeled for the probability of antibiotic use. The differential effect of antibiotics on OS (ie, the "true antibiotic effect") within the ICI versus other cancer treatment modality cohort was modeled using an interaction term for antibiotic use × ICI use. The survivor function of PROC PHREG was used to visualize absolute risk estimates over time. All statistical analyses were performed using SAS software, V.9.4 (PROC PHREG and PROC LOGISTIC).

RESULTS

Patient characteristics

In total 4534 patients were included in this study. They were equally distributed between underlying malignant diseases (see table 1). In both ICI and TKI cohorts, roughly 10% were current users of antibiotics (within 30 days before the start of cancer treatment). As expected, patients with NSCLC were more likely to be exposed to antibiotics compared with patients with melanoma, in all four cohorts. Overall, current antibiotics users were more likely to be concomitantly exposed to other comedications, which may reflect a relatively poor health status. Median follow-up was 10.0 months (IQR=19.2 months) for the entire study population. Baseline characteristics stratified by underlying malignancy are available in online supplemental file 1.

Synergistic interaction between antibiotics and ICI on overall survival

The results in table 2 demonstrate a lack of synergistic interaction between current antibiotic use and ICI therapy in relation to OS. Within the ICI cohort, the results of the propensity score adjusted multivariable analysis show a significant decrease in OS among antibiotic users compared with non-users, when the antibiotics were used up to 365 days before the start of ICI therapy (see also figure 1). This effect diminished among patients exposed to antibiotics more than 365 days before the start of anticancer treatment. However, similar magnitudes in OS decline were found within the TKI cohort and also within the chemoimmunotherapy and chemotherapy cohorts. The similar OS decline in the ICI and TKI cohorts was reflected by the synergy index in bold in table 2: no synergistic interaction between current antibiotic use and ICI was found, yielding a synergy index of 0.96 (95% CI (0.70 to 1.31)) (see also figure 2). Moreover, the observed inverse association between antibiotic use and ICI therapy was similar in the first 3 months (HR=1.19 (95% CI (0.88 to 1.62)) as compared with the period thereafter (HR=1.28 (95% CI (1.04 to 1.59)). As ICI therapy typically exerts its effect after a couple of months, this finding underlines the lack of a potential causal relationship. Finally, exposure to oral or topical antibiotics concurrent to cancer treatment showed a similar lack of differential effect between the TKI and ICI cohort.

Effect modification by underlying malignant disease, type of antibiotic and concomitant medication use

Synergy indexes of current antibiotic use and ICI therapy, stratified by underlying malignant diagnosis (melanoma or NSCLC), route of antibiotic administration (oral or intravenous) or concomitant use of systemic corticosteroids (yes or no) are shown in table 3. No detrimental synergistic interaction was found in any of the studied strata for either the primary endpoint OS or the composite secondary endpoint of death or cancer treatment switch.

DISCUSSION AND CONCLUSION

Our study suggests that antibiotic therapy at cancer treatment initiation does not detrimentally impact ICI therapy response in melanoma and NSCLC. The magnitude of decreased OS in antibiotic users was similar between individuals on ICI therapy (HR=1.28; 95% CI 1.07 to 1.52) and those on TKIs (HR=1.20; 95% CI 0.92 to 1.57; synergy index=0.96; 95% CI 0.71 to 1.31). In addition, time-dependent analyses demonstrated that this decrease in OS was already observed in the first 3 months, all in all indicative for confounding by indication rather than a true pharmacological interaction between antibiotic and ICI therapy.

Most previous studies did not look at the differential effect of antibiotics on OS between various cancer

Table 1 Baseline characteristics of patients with metastatic melanoma and non-small cell lung cancer, stratified by ICI/non-ICI treatment and co-use of systemic antibiotics

	Immune checkpoint inhibitors		Tyrosine kinase inhibitors		Chemoimmunotherapy		Chemotherapy	
	N=1,908		N=817		N=193		N=1,616	
	Antibiotics	No antibiotics	Antibiotics	No antibiotics	Antibiotics	No antibiotics	Antibiotics	No antibiotics
	N=174	N=1,734	N=82	N=735	N=29	N=164	N=164	N=1,452
Age (years, median, SD)	6.5 (11.6)	63 (12.0)	68 (12.1)	64 (13.1)	62 (9.2)	62 (9.6)	61.5 (12.8)	65 (11.3)
Follow-up (years, median, SD)	0.5 (1.0)	0.8 (1.2)	0.5 (1.2)	0.8 (1.5)	0.4 (0.6)	0.8 (1.1)	0.5 (1.7)	1 (1.6)
Males	77 (44%)	784 (45%)	41 (50%)	431 (59%)	20 (69%)	84 (51%)	78 (48%)	719 (50%)
Underlying malignancy								
Malignant melanoma	70 (40%)	989 (57%)	21 (26%)	256 (35%)	2 (7%)	31 (19%)	51 (31%)	526 (36%)
Non-small cell lung cancer	104 (60%)	745 (43%)	61 (74%)	479 (65%)	27 (93%)	133 (81%)	113 (69%)	926 (64%)
Liver metastasis	6 (3%)	168 (10%)	3 (4%)	21 (3%)	1 (3%)	4 (2%)	2 (1%)	17 (1%)
Brain metastasis	7 (4%)	108 (6%)	4 (5%)	40 (5%)	0 (0%)	7 (4%)	9 (5%)	40 (3%)
Lactate dehydrogenase (IU/L, median, SD)	193 (551)	196 (225)	231 (646)	212 (252)	238 (167)	183 (130)	209 (505)	189 (225)
Neutrophil lymphocyte ratio (median, SD)	5.2 (9.2)	3.4 (5.0)	8.3 (14.6)	4.3 (6.9)	6.8 (5.7)	4.5 (4.6)	5.6 (9.2)	4.4 (14.0)
Drug use in previous 30 days								
Systemic corticosteroids	65 (37%)	256 (15%)	27 (33%)	147 (20%)	21 (72%)	71 (43%)	83 (51%)	432 (30%)
Proton pump inhibitors	64 (37%)	245 (14%)	39 (48%)	147 (20%)	7 (24%)	34 (21%)	79 (48%)	380 (26%)
Antidepressants	11 (6%)	32 (2%)	6 (7%)	20 (3%)	1 (3%)	10 (6%)	11 (7%)	39 (3%)
Opioids	70 (40%)	298 (17%)	31 (38%)	139 (19%)	19 (66%)	45 (27%)	83 (51%)	372 (26%)
Statins	20 (11%)	95 (5%)	10 (12%)	47 (6%)	7 (24%)	14 (9%)	131 (80%)	24 (2%)
RAAS inhibitors	22 (13%)	81 (5%)	11 (13%)	53 (7%)	4 (14%)	11 (7%)	22 (13%)	150 (10%)

ICI, immune checkpoint inhibitors; RAAS, renin-angiotensis-aldosterone system.

Table 2 Overall survival with antibiotic use compared with non-use expressed as a synergy factor between ICI/TKI use

	All-cause mortality								
	Immune checkpoint inhibitors (ICI)		Tyrosine kinase inhibitors (TKI)		Effect of antibiotic use	Chemoimmunotherapy		Chemotherapy	
	N	aHR (95% CI)	N	aHR (95% CI)	Synergy index (ICI vs TKI)	N	aHR (95% CI)	N	aHR (95% CI)
No antibiotics	1,314	Reference	648	Reference	–	101	Reference	1,212	Reference
Antibiotic use prior to start of cancer therapy									
≤30 days before	174	1.26 (1.04 to 1.51)	82	1.24 (0.95 to 1.62)	**0.96 (0.70 to 1.31)**	29	1.26 (0.76 to 2.08)	164	1.06 (0.86 to 1.30)
31–90 days before	127	1.51 (1.22 to 1.86)	37	1.18 (0.77 to 1.80)	**1.13 (0.72 to 1.80)**	23	1.56 (0.96 to 2.56)	102	0.77 (0.58 to 1.00)
91–365 days before	189	1.47 (1.24 to 1.76)	31	1.68 (1.11 to 2.57)	**0.83 (0.53 to 1.31)**	33	1.81 (1.15 to 2.84)	67	1.48 (1.10 to 1.99)
>365 days before	104	1.03 (0.81 to 1.30)	19	0.90 (0.53 to 1.52)	**1.17 (0.67 to 2.05)**	7	1.21 (0.49 to 3.02)	71	0.98 (0.71 to 1.34)
Antibiotic use at any time during cancer therapy									
Oral	945	0.84 (0.76 to 0.92)	376	0.83 (0.71 to 0.98)	**0.92 (0.78 to 1.09)**	130	0.69 (0.54 to 0.89)	812	0.73 (0.66 to 0.81)
Topical	88	0.75 (0.57 to 1.00)	63	1.03 (0.67 to 1.38)	**0.67 (0.45 to 1.00)**	15	0.86 (0.50 to 1.47)	97	1.36 (1.01 to 1.82)

aHR fully adjusted for age, sex, tumor stage, number/site of metastases, underlying malignant disease, comorbidities, concomitant drug use, baseline organ function, C reactive protein, lactate dehydrogenase, white blood cell count and neutrophil lymphocyte ratio.
aHR, adjusted HR.

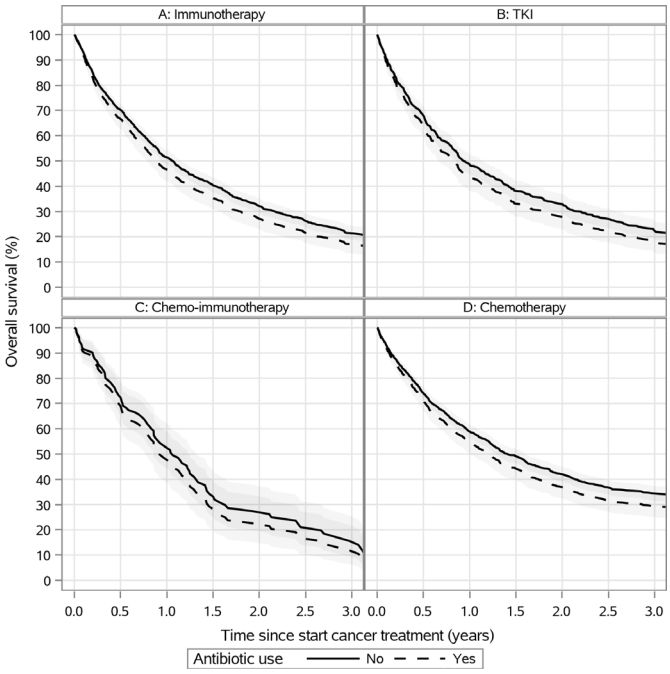

Figure 1 Propensity-adjusted overall survival for current antibiotic users versus non-users in immune checkpoint inhibitor, TKI, chemoimmunotherapy and chemotherapy cohorts. TKI, tyrosine kinase inhibitor.

treatment strategies and could therefore not study confounding by indication. In line with most of these studies, we found that antibiotic use was significantly associated with a detrimental impact on ICI therapy response in patients with melanoma and NSCLC. However, in our study, this same trend in decreased OS was seen in a similar cohort of patients with TKI-treated melanoma and NSCLC, strongly suggesting confounding by indication as opposed to a true causal relationship between antibiotics

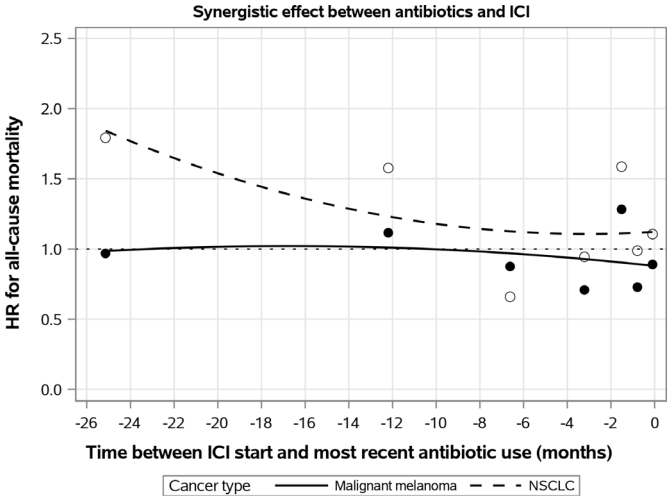

Figure 2 Synergy factor HR for all-cause mortality against time between cancer treatment start and most recent antibiotic use in ICI cohort and tyrosine kinase inhibitor cohort, stratified by underlying malignant disease. ICI, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer.

and ICI therapy. Indeed, observational studies typically find that individuals taking antibiotics are generally less healthy, which is difficult to adjust for in these types of studies. As an example, a large Swedish cohort study was conducted studying the relationship between antibiotic use and onset of asthma.[14] They found strong suggestions of confounding by indication and reverse causation, as antibiotic use was found to be a proxy for respiratory tract infections and poor health status. Careful analyses, such as a time-dependent approach and interaction analyses, are therefore warranted when looking at antibiotic use and clinical outcomes in observation studies. Cortellini *et al* used a similar approach as in our study, but used a chemotherapy cohort as a reference population.[12] Their analysis suggested a differential effect between ICI therapy (HR 1.47) and chemotherapy (HR 1.23), but the interaction did not reach statistical significance (p=0.111). Moreover, individuals on chemotherapy may particularly benefit from antibiotic use as it may protect against infections in patients suffering from neutropenia.[15 16] Any differential effect might therefore be overestimated, because of this additional beneficial effect of antibiotics, that is, probably more apparent among individuals on chemotherapy.

Our timing analyses confirmed that the association between antibiotics and ICI therapy is most likely not causally related. Antibiotic use 91–365 days before the start of ICI treatment was significantly associated with decreased OS. There is however no biological plausibility for any effect to take place when using antibiotics so long before the start of ICI treatment. The patient's microbiome would have recovered from the antibiotic treatment by the time the patient starts the ICI regimen, as is demonstrated looking at gut microbiomics in healthy individuals.[17 18] Moreover, our results show there is already an effect of antibiotic use on ICI efficacy in the first 3 months after treatment initiation. In randomized clinical trials, the effect of ICIs on OS usually does not arise within these first 3 months, which means any effect of antibiotics within this early time frame is probably not mediated via the ICI immune reaction.

The hypothesized mechanism lies in potential dysbiosis of the gut microbiome caused by antibiotic use, defined as compositional and functional alterations. Dysbiosis of the gut microbiome has been linked to perturbation of both the innate and adaptive immune system.[19] Studies of the gut microbiota in healthy adults shows perturbation of the microbiome within a few days after the start of antibiotic treatment, but also shows recovery of the microbiome to pre-antibiotic treatment state after 1.5–2 months.[17 18] Almost all data on gut microbiome status following antibiotic treatment are derived from healthy subjects, making it difficult to draw conclusions for a cohort of cancer-treated patients. However, in our cohort of ICI-treated patients it takes more than 1 year for the effect of antibiotics on the ICI-response to wear off, which seems a very long time for the microbiome to recover compared with healthy individuals. The interaction term analysis indicated the microbiome dysbiosis is

Table 3 Overall survival and cancer treatment switch with antibiotic use compared with non-use expressed as a synergy factor between ICI/TKI use

	All-cause mortality	Death or cancer treatment switch
	Effect of antibiotic use	Effect of antibiotic use
	Synergy index (ICI vs TKI)	Synergy index (ICI vs TKI)
No antibiotics		
Antibiotic use prior to start of cancer therapy		
≤30 days before	0.96 (0.70 to 1.31)	0.93 (0.68 to 1.26)
By underlying malignant diagnosis		
Malignant melanoma	0.85 (0.49 to 1.48)	0.75 (0.43 to 1.31)
Non-small cell lung cancer	0.97 (0.70 to 1.34)	0.92 (0.63 to 1.33)
By route of administration		
Oral	0.85 (0.63 to 1.15)	0.90 (0.64 to 1.25)
Intravenous	1.23 (0.58 to 2.60)	0.89 (0.41 to 1.92)
By concomitant use of systemic corticosteroids		
No	1.00 (0.68 to 1.46)	1.08 (0.74 to 1.59)
Yes	0.86 (0.57 to 1.30)	0.65 (0.38 to 1.10)

ICI, immune checkpoint inhibitor; TKI, tyrosine kinase inhibitor.

recovered from within a time frame more comparable to healthy individuals, with antibiotic treatment started within 30 days before the start of ICI treatment not having any impact. Keeping in mind the fact that ICI treatment only elicits a response a few months after the start of treatment, this would allow for the microbiome to recover in a timely fashion.

Any detrimental effect from the use of antibiotics may be dependent on the actual cancer treatment strategy, either by a direct effect (immune driven tumor clearance varies between immunotherapy, chemotherapy, TKI and chemoimmunotherapy) as well as by an indirect effect (underlying cancer disease may have different clinical outcomes). Although Cortellini and colleagues could not demonstrate a significant effect of antibiotics in their chemoimmunotherapy study (adjusted HR (aHR) for OS: 1.42, 95% CI 0.91 to 2.22) versus within immunotherapy alone (aHR 1.42, 95% CI 1.13 to 1.79), the overall estimates for OS were, in line with our results, very similar (both 1.42), suggesting that this difference may—at least in part—be driven by lower number of individuals (302 chemoimmunotherapy vs 950 immunotherapy alone).[12 19] Strengths of this large study include a large sample size, its multimodal bias approach (rich propensity score adjustments, the inclusion of a TKI control cohort) and careful timing patterns to assess potential causality. Propensity scores allowed us to adjust for a large range of confounders, not only age, gender, tumor status and underlying malignancy, but also use of other co-medications in the time frame studied, organ function at baseline and advanced disease stage. This study is the first to use a control cohort of TKI-treated patients for interaction analysis, allowing us to further adjust for bias introduced by factors for which data was not available. TKI-treated patients are more comparable to ICI-treated patients, than chemo-treated patients (used as control cohort in previous studies). Also, there is no calendar-time bias as melanoma and NSCLC have been treated with ICI's and TKI's in the same time period. Changes in prescription of antibiotics over the years (prescribing less often, prescribing different regimens, etc) therefore has no effect on our two cohorts, which it would when using a chemo-treated cohort as control.

A shortcoming of our study is that we did not have data on temporary drug suspension due to drug–drug interaction. We also did not have data on objective response/PFS, which could be helpful in further exploring a possible mechanism between antibiotic use and response to anticancer treatment. However, as we found no differential trend in OS, a further exploration based on objective response is therefore probably of limited additional value. To partially overcome this, we assessed a composite endpoint of therapy switch and death to mimic progression-free/toxicity-free survival. The results showed the same trend as for OS. Second, the cohort was unfortunately not large enough to further stratify by specific antibiotic classes. This could be helpful (1) to allow clinicians to base their decisions on antibiotic subtypes and (2) to further explore hints for causality. Third, although we carefully adjusted for a substantial list of potential confounders using a propensity score approach, we cannot rule out the possibility of residual bias by unknown factors. Lastly, there may be an immunologic factor in the mechanism of response to TKIs, leaving it potentially vulnerable to antibiotic interaction (ie, any detrimental, immunologically driven effect of antibiotics may also have been present among TKI users). However, we believe that this effect would be much smaller than

what can be expected in ICI-treated patients, and therefore hypothesize that the effect should still be differential if antibiotics indeed confer an immunologically detrimental effect on ICI therapy.

In conclusion, our study strongly suggests that there is no detrimental association between antibiotic use and ICI therapy when looking at OS in individuals with malignant melanoma or NSCLC. The frequently observed inverse association between antibiotics and ICI response in previous studies is most likely driven by confounding by indication, which was confirmed by our reference TKI cohort and time-dependent approach. Although clinicians should remain judicious in the use of antibiotics in general, concomitant use of ICI therapy does not seem to form a contraindication and continued use is warranted in justified underlying diseases.

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Provenance and peer review Not commissioned; externally peer reviewed.

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