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Comparative analysis of antibiotic exposure association with clinical outcomes of chemotherapy versus immunotherapy across three tumour types

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ABSTRACT

Background In solid tumours, antibiotic use during immune checkpoint inhibitor (ICI) treatment is associated with shorter survival. Following allogeneic haematopoietic cell transplantation (allo-HCT), antibiotic-induced gut microbiome alterations are associated with risk of relapse and mortality. These findings suggest that the gut microbiota can modulate antitumour immune response across tumour types, though it is not clear if the impact on outcomes is specific to immune therapy. An important limitation of previous studies is that the analysis combined all antibiotic exposures irrespective of the antibiotic spectrum of activity. Whether antibiotic exposure during induction chemotherapy in acute myeloid leukaemia (AML) affects risk of relapse is also unknown.

Patients and methods We performed a single-centred retrospective analysis of antibiotic exposures in metastatic/advanced non-small cell lung cancer (NSCLC) and renal cell cancer (RCC) receiving ICI and newly diagnosed AML patients receiving induction chemotherapy achieving a complete remission 1. Antibiotic use within 4 weeks before and 6 weeks after the ICI initiation were included. In AML patients, antibiotic exposures between days 1 and 28 of induction were collected. Antibiotics were a priori stratified based on spectrum of activity. Primary outcomes of interest were progression-free survival (PFS), overall survival (OS) in NSCLC and RCC and relapse-free survival (RFS) in AML.

Results 140 patients with NSCLC, 55 with RCC and 143 with AML were included. In multivariable analysis, PFS and OS were shorter in NSCLC patients who received broad-spectrum anti-anaerobes (PFS, HR=3.2, 95% CI 1.6 to 6.2, p<0.01; OS, HR=1.7, 95% CI 0.8 to 3.6, p=0.19) or 'other' antibiotics (vancomycin-predominant) (PFS, HR=2.4, 95% CI 1.3 to 4.6, p<0.01; OS, HR=2.4, 95% CI 1.2 to 4.7, p=0.01). In RCC, patients who received penicillins/penicillin-class/early-generation cephalosporins had shorter PFS (HR=3.6, 95% CI 1.7 to 7.6, p<0.01) but similar OS (p=0.37). In the AML cohort, none of the exposures were associated with RFS.

Conclusion In contrast to AML, antibiotic exposures in solid tumours affected clinical outcomes. The presence of an allogeneic effect (allo-HCT) or an augmented immune system (checkpoint blockade) may be necessary for microbiota mediation of relapse risk.

Key questions

What is already known?

- Antibiotics have been previously shown to negatively affect treatment outcomes of immune checkpoint blockers in solid tumours and associated with risk of leukaemia relapse post-allogeneic haematopoietic cell transplant.

What does this study add?

- We tested the effect of exposure to different antibiotic subclasses based on spectrum of activity on clinical outcomes of immune checkpoint blocker in non-small cell lung cancer (NSCLC) and renal cell cancer (RCC) and tested the risk of relapse-free survival (RFS) in acute myeloid leukaemia (AML) following induction chemotherapy.
- We found that NSCLC patients who received broad-spectrum anti-anaerobes (most commonly piperacillin-tazobactam) or intravenous vancomycin had shorter progression-free survival (PFS) and overall survival.
- RCC patients who received penicillins, penicillin-class or early-generation cephalosporins had the greatest impact on PFS. In AML, none of the antibiotic exposures were associated with RFS.

How might this impact on clinical practice?

- Antibiotics-induced gut microbiome dysbiosis can influence clinical outcomes of immunotherapy in solid tumours. The impact might be dependent on the type of antibiotic used and its spectrum of activity.

INTRODUCTION

The commensal gut microbiota has been increasingly recognised as a key constituent orchestrating the activity and repertoire of host immunity.[1–5] Antibiotic use among

cancer patients is common and can lead to disruption of the microbiome community structure and function ('dysbiosis'). In allogenic haematopoietic cell transplant (allo-HCT) recipients, early post-transplant gut microbiota alterations predict mortality.[6] Similarly, several studies have shown that antibiotic use just before initiation and during immune checkpoint inhibitors (ICI) use was associated with shorter progression-free survival (PFS) and overall survival (OS) in patients with non-small cell lung cancer (NSCLC), renal cell cancer (RCC), urothelial cancer and melanoma.[7–26] In contrast to solid tumours treated with ICI, the success of cytotoxic chemotherapy in acute myeloid leukaemia (AML) depends on non-immune direct cytotoxicity, and thus, would not be expected to be changed by potential microbiota-mediated immune effects. However, the intestinal microbiota can modify antineoplastic effects of some chemotherapeutic agents.[27 28] We therefore tested the effect of antibiotic exposure on immunotherapy outcomes in NSCLC and RCC and compared it with outcomes of AML patients receiving induction chemotherapy.

METHODS

NSCLC and RCC cohorts

After Institutional Review Board (IRB) approval, we retrospectively identified consecutively diagnosed patients with relapsed or refractory advanced or metastatic NSCLC and RCC who received any single-agent ICI at any of the M-Health Fairview health system sites in the Minneapolis region between May 2015 and December 2017. Follow-up was completed as of August 2019. Patients who received at least two doses of ICI as a part of standard-of-care treatment were included. Patients receiving combination chemoimmunotherapy were excluded. Data on systemic antibacterial antibiotic use (inpatient or outpatient; oral or intravenous; any duration) within 1 month before and 6 weeks after the first dose of ICI were reviewed. Only patients surviving at least 6 weeks after the first ICI dose were included in the analysis. Antibiotic use was classified as any antibiotic use (yes vs no). A subclassification was performed for different antibiotic classes: group 1 (fluroquinolones: levofloxacin, ciprofloxacin, macrolides: azithromycin, tetracyclines: doxycycline, minocycline); group 2 (broad-spectrum anti-anaerobes (AA): piperacillin-tazobactam, clindamycin, metronidazole, meropenem); group 3 (third-generation and fourth-generation cephalosporins: cefdinir, cefpodoxime, ceftazidime, ceftriaxone, cefepime); group 4 (penicillins, penicillin-class or first-generation and second-generation cephalosporins: cefazolin, cephalexin, cefuroxime, amoxicillin, penicillin, ampicillin, nafcillin, oxacillin, ampicillin-sulbactam and group 5 (others: vancomycin, nitrofurantoin, rifampin, rifaximin, tobramycin, trimethoprim-sulfamethoxazole). PFS was defined as the time from first dose of ICI until disease progression or death as assessed by Response Evaluation Criteria in Solid Tumors (V.1.1). OS was measured as time from first dose of ICI to death.

AML cohort

After IRB approval, we retrospectively identified newly diagnosed, consecutive AML patients achieving a complete remission (CR1) with one or two rounds of standard-of-care intensive chemotherapy (including the 7+3 regimen: cytarabine plus daunorubicin or idarubicin) at the University of Minnesota Medical Center between December 2011 and June 2018. Antibacterial antibiotic exposures (oral or intravenous; prophylactic or therapeutic; any duration) between day 1 of induction and day 28 were extracted. The recommended antibiotic prophylaxis was levofloxacin, acyclovir and an azole. Cefepime was the recommended front-line empiric antibiotic for neutropenic fever. Since antibiotic types and exposure patterns are different between AML and NSCLC or RCC, we used a different classification system herein: fluoroquinolones (FQN): ciprofloxacin, levofloxacin; AA: piperacillin-tazobactam, clindamycin, metronidazole, imipenem, meropenem; third-generation or higher-generation cephalosporins (CPN3+): cefepime, ceftriaxone, ceftazidime; intravenous vancomycin (IV vanc) and oral vancomycin (oral vanc). Relapse-free survival (RFS) was measured as the time from achieving CR1 to disease relapse or death.

Statistical analysis

NSCLC and RCC cohorts

The primary end points were PFS per and OS at 1 year. The secondary end point was clinical benefit rate (CBR) defined as the proportion of patients with best response as CR, partial response or stable disease. Analysis was performed using any antibiotic exposures and in predefined antibiotic subgroups based on different antibiotic subclasses. Fisher's exact test was used for univariate analysis of CBR. Multivariable logistic regression was used to assess the independent association of antibiotics with CBR. Kaplan-Meier (K-M) curves were used to estimate OS and PFS. Cox regression was used to assess the independent association of antibiotics with PFS and OS. Prespecified potential confounders in the NSCLC cohort were age, gender, Eastern Cooperative Oncology Group (ECOG) performance status (0–1 vs 2+), histology (adenocarcinoma vs squamous vs other) and prior lines of therapy (<2vs ≥2). The same variables were used in the RCC cohort except that histology was excluded as 96% patients had clear cell histology.

AML cohort

The primary end point was RFS. Secondary end points included response (CR vs primary induction failure (PIF)), OS, relapse and non-relapse mortality (NRM). OS, relapse and NRM were measured from day 1 of chemotherapy in time-to-event analysis. Covariates for multivariable analysis included predetermined covariates important for relapse and RFS: age, AML risk (based on ELN-2017)[29] and number of inductions (1 vs 2).

All reported p values were two-sided. SAS V.9.4 (SAS Institute) and R V.3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for analyses.

Table 1 Patient characteristics NSCLC and RCC cohort

	RCC	NSCLC
N	55	140
Gender: male	40 (73%)	67 (48%)
Age		
Median (range)	62 (23–86)	66 (39–92)
Race	62 (23–86)	66 (39–92)
Caucasian	52 (95%)	131 (93%)
African American	2 (4%)	3 (2%)
Others	1 (2%)	6 (3%)
Pathology		
Adenocarcinoma	0	91 (64%)
Squamous	0	41 (29%)
Others	0	8 (6%)
Clear cell	53 (96%)	0
Papillary	2 (4%)	0
Drug		
Nivolumab	51 (93%)	123 (88%)
Pembrolizumab	0	11 (8%)
Others	4 (7%)	6 (4%)
ECOG		
0–1	36 (66%)	99 (71%)
≥2	19 (34%)	36 (25%)
Missing	0	5 (4%)
Number of doses		
Median (range)	8 (2–63)	7 (2–89)
Ongoing doses		
NA	1 (2%)	0
Yes	11 (20%)	12 (9%)
No	43 (78%)	128 (91%)
Months of therapy		
Median (range)	5 (1–35)	3.9 (1–47)
Previous lines of therapy		
Median (range)	1 (0–5)	1 (1–4)
Smoking status		
Never	Not available	15 (11%)
Former	Not available	88 (63%)
Current	Not available	30 (21%)

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer; RCC, renal cell cancer.

RESULTS

NSCLC and RCC cohorts

Median follow-up was 11 months in NSCLC and 18.7 months in RCC. Of note, 148 metastatic NSCLC patients and 55 metastatic RCC patients received at least two doses of ICI. Eight patients with NSCLC were excluded from the final analysis because they died within 6 weeks of the first ICI dose. Final analysis included 140 patients with metastatic NSCLC and 55 with metastatic RCC. Table 1 shows clinical characteristics of the patient population. Median age was 66 years (range 39–92) and 62 years (range 23–86) in NSCLC and RCC cohorts, respectively. Almost all patients were Caucasian (93% of NSCLC; 95% of RCC) and most had adenocarcinoma (64% of NSCLC) or clear cell histology (93% of RCC). Most patients received nivolumab in the second or later line setting (88% of NSCLC and 93% of RCC). Of note, 54/140 (39%) and 24/55 (44%) patients received at least one antibiotic dose within 1 month before or 6 weeks after the first dose of ICI in NSCLC and RCC cohorts, respectively (online supplementary table S1).

The results of univariate analysis for NSCLC are shown in online supplementary table S2. In multivariable analysis, any antibiotic use was not with PFS (median 3.5 vs 3.9 months, p=0.44) and OS (median 17 vs 11 months, p=0.34) (figure 1). Patients who received AA had shorter PFS (median 1.5 vs 4.0 months, HR=3.2, 95% CI 1.6 to 6.2; p<0.01) and OS (median 3.0 vs 12.0 months; HR=1.7, 95% CI 0.8 to 3.6; p=0.19) (figure 2 and table 2). Patients who received 'other' antibiotics had shorter PFS (median 2.0 vs 4.0 months, HR=2.4, 95% CI 1.3 to 4.6; p<0.01) and OS (median 2.0 vs 12.0 months; HR=2.4, 95% CI 1.2 to 4.7; p=0.01) (figure 2 and table 2). Antibiotic groups 1, 3 and 4 were not associated with PFS or OS (online supplementary table S1).

The results of univariate analysis for RCC are shown in (online supplementary table S3) . In multivariable analysis, any antibiotic use was associated with shorter PFS (median 2.7 vs 4.2 months, HR=2.7, 95% CI 1.3 to 5.9; p=0.01) and OS (median 17 vs 22 months, HR=4.2, 95% CI 1.5 to 12.2; p<0.01) (figure 1). Patients who received penicillins, penicillin-class or first-generation and second-generation cephalosporins had shorter PFS (median 2.7 vs 4.2 months, HR=3.6, 95% CI 1.7 to 7.6; p<0.01) but similar OS (p=0.37) (table 3 and figure 2). None of the other antibiotic groups were associated with PFS or OS (online supplementary table S3).

In multivariable analysis, in the NSCLC cohort, CBR was similar in patients who received any antibiotics compared with patients who were antibiotic-free (49% vs 54%; p=0.96). In contrast, patients who received AA or 'other' antibiotics had lower CBR (11% vs 55%; p=0.06and 20% vs 54%; p=0.04), respectively. Results of CBR analysis in NSCLC are shown in online supplementary table S4. In the RCC cohort, patients who received any antibiotics had lower CBR compared with patients who were antibiotic-free (30% vs 59%; p=0.10). In addition, patients who received penicillins, penicillin-class or first-generation and second-generation cephalosporins had lower CBR (20% vs 57%; p<0.01). Results of CBR analysis in RCC are shown in online supplementary table S5. Indications for antibiotics in NSCLC and RCC are shown in online supplementary table S6.

AML cohort

Of note, 143 patients achieved CR1 and 27 patients had PIF. First, we analysed the CR1 subgroup. Median (range) age was 59 (19–75) years and 83 (58%) were men. ELN-2017 risk was poor, intermediate and favourable in 29 (20%), 80 (56%) and 34 (24%) patients,

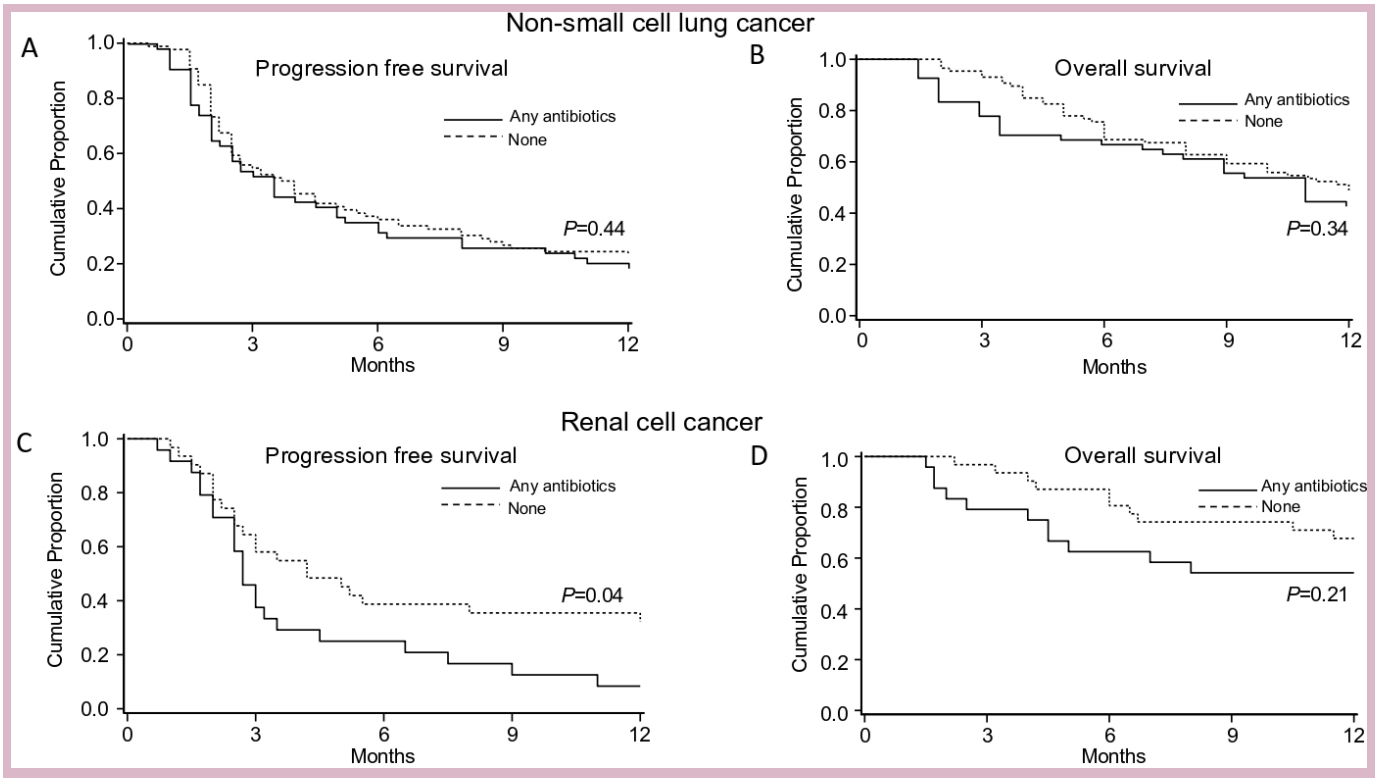

Figure 1 Kaplan-Meier curves of progression free survival and overall survival in non-small cell lung cancer and renal cell cancer with any antibiotic use.

respectively. Forty-six (32%) patients received two inductions (table 4). All patients received antibacterial antibiotics for prophylaxis and/or treatment. Exposure to FQN occurred in 122 (85%), AA antibiotics in 116 (81%) patients, CPN3+ in 110 (77%) patients, IV vanc in 108 (76%) patients and oral vanc in 14 (10%) of patients. None of the antibiotic groups were associated with RFS (FQN: HR 0.75, 95% CI 0.42 to 1.36, p=0.35; AA antibiotics: HR 1.02, 95% CI 0.59 to 1.76, p=0.94; CPN3+: HR 1.29, 95% CI 0.77 to 2.16, p=0.34; IV vanc: HR 1.1, 95% CI 0.67 to 1.82, p=0.71; oral vanc: HR 0.63, 95% CI 0.28 to 1.45, p=0.28) (table 5). Censoring at the time of

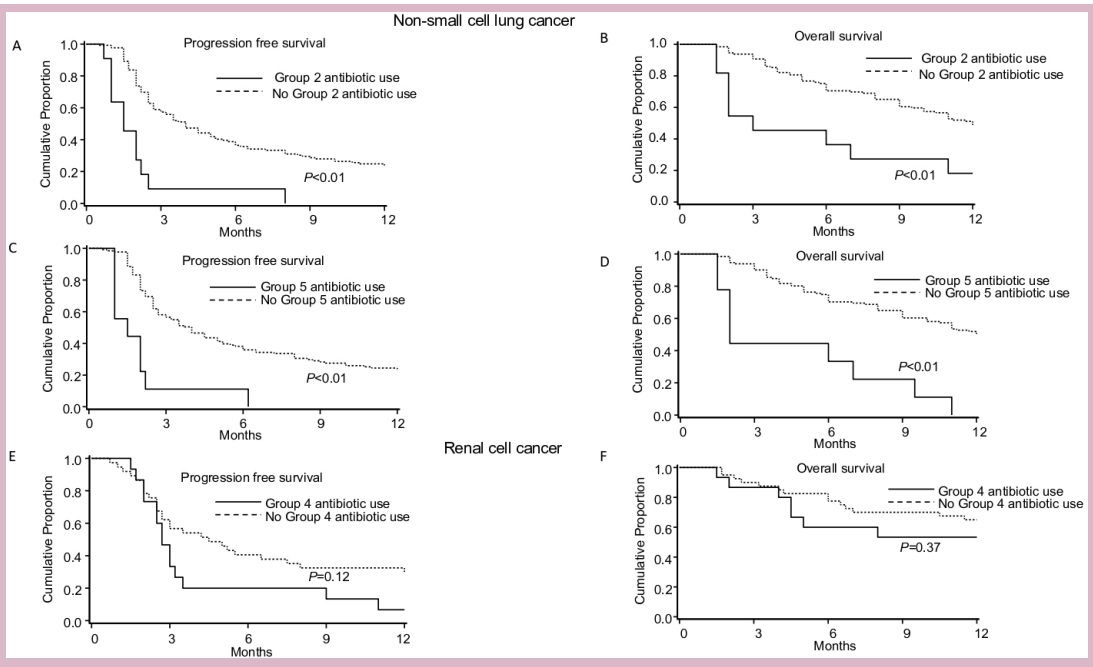

Figure 2 Kaplan-Meier curves of progression free survival and overall survival in non-small cell lung cancer and renal cell cancer with specific antibiotic class based on spectrum of activity.

Table 2 Multivariable analysis of PFS and OS in group 2 and group 5 antibiotics in NSCLC

	Group 2 (NSCLC)				Group 5 (NSCLC)			
	PFS, HR (95% CI)	P	OS, HR (95% CI)	P	PFS, HR (95% CI)	P	OS, HR (95% CI)	P
	3.2 (1.6 to 6.2)	<0.01	1.7 (0.8 to 3.6)	0.19	2.4 (1.3 to 4.6)	<0.01	2.4 (1.2 to 4.7)	0.01
ECOG (0–1 vs 2+)	1.6 (1.1 to 2.5)	0.02	2.6 (1.6 to 4.3)	<0.01	1.6 (1.1 to 2.5)	0.02	2.6 (1.2 to 4.7)	<0.01
Histology		NS		NS		NS	NS	
A vs S	1.0 (0.7 to 1.6)		1.3 (0.7 to 2.1)		1.1 (0.7 to 1.7)		1.2 (0.7 to 2.1)	
A vs others	1.3 (0.6 to 2.9)		1.3 (0.5 to 3.4)		1.4 (0.6 to 3.1)		1.4 (0.6 to 3.6)	
Prior therapy (0–1 vs 2+)	0.9 (0.6 to 1.2)	NS	0.7 (0.4 to 1.2)	NS	0.8 (0.5 to 1.2)	NS	0.7 (0.4 to 1.2)	NS

A, adenocarcinoma; ECOG, Eastern Cooperative Oncology Group; NS, non-significant; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; S, squamous.

allo-HCT did not change the results. Figure 3 shows K-M curves for RFS.

Twenty-seven patients had PIF. There were no differences between CR1 and PIF rates in patients with or without exposure to each antibiotic group except IV vanc, where PIF was higher in the exposed group (19% vs 3%; p=0.018). There was no association of OS, relapse and NRM with exposure to any of the antibiotic groups (table 5).

DISCUSSION

In this study, we compared the association of antibiotic use with clinical outcomes among two distinct cohorts, that is, patients with solid tumours (RCC and NSCLC) receiving immunotherapy and patients with AML receiving induction chemotherapy. We systematically evaluated the impact of exposure to different antibacterial antibiotic classes and explored the effect of exposure duration on clinical outcomes. We found that in contrast to chemotherapy-treated AML, exposure to some antibiotic groups during immunotherapy-treated NSCLC/RCC was associated with clinical outcomes. To our knowledge, this is the first study to test the association of antibiotic use with RFS in the non-transplant setting. Previous studies in allo-HCT recipients showed an association between early post-transplant gut microbiota alterations and transplant-related mortality[30] or relapse of haematological malignancies.[6] Our study only looked at the time period during induction chemotherapy which may be one reason for the observed difference. Alternatively, our study may suggest that for microbiota-mediated increased risk of relapse, an augmented immune system by allogeneic effect may be necessary. Further studies directly evaluating microbiome profile during chemotherapy and after allo-HCT may be informative.

In contrast to previously published studies in NSCLC,[8–13 16 21–23 26 31] our study did not show an association of antibiotic use with PFS or OS. One reason for this discrepancy could be related to the differences in the chosen window of antibiotic exposure among different studies. With respect to NSCLC, our study is the only one to assess antibiotic use during the window of 10 weeks around ICI use. We chose the 6-week post-ICI initiation window to allow at least 2–3 doses of nivolumab, the most commonly used ICI in the study population. In comparison, other studies in NSCLC assessed a range of different antibiotic exposure windows. Some of the studies tested only during the period before immunotherapy (up to 2 months).[12 22 26] Others chose arbitrary time windows anywhere from up to 2 months before beginning immunotherapy[7–11 13 21 23 26] or through the

Table 3 Multivariable analysis of PFS in group 4 antibiotics in RCC

	HR (95% CI)	P
Variables	3.6 (1.7 to 7.6)	<0.01
Age	0.9 (0.92 to 0.98)	<0.01
Gender	1.0 (0.5 to 2.1)	NS
ECOG (0–1 vs 2+)	8.5 (3.8 to 18.8)	<0.01

ECOG, Eastern Cooperative Oncology Group; NS, non-significant; PFS, progression-free survival; RCC, renal cell carcinoma.

Table 4 Characteristics of the AML cohort who achieved first complete remission

Total	143
Males, N (%)	83 (58%)
Age (years), median (range)	59 (19–75)
AML type, n (%)	
t-AML/s-AML	16 (11%)
AML-MDS/MPN/haematological disorder	27 (19%)
De-novo AML	100 (59%)
ELN-2017 risk, n (%)	
Favourable	34 (24%)
Intermediate	80 (56%)
Adverse	29 (20%)
Re-induction, n (%)	46 (32%)

AML, acute myeloid leukaemia; AML-MDS, AML with myelodysplastic changes; s-AML, secondary AML; t-AML, therapy-related AML.

Table 5 Analysis of outcomes in the AML cohort

Antibiotic	OS, HR (95% CI), p	RFS, HR (95% CI), p	Relapse, HR (95% CI), p	NRM, HR (95% CI), p
FQN (yes vs no)	0.95 (0.47–1.92), 0.89	0.65 (0.35 to 1.2), 0.17	0.89 (0.43 to 1.84), 0.76	0.65 (0.18 to 2.3), 0.5
Risk				
Favourable vs high		0.64 (0.33 to 1.26), 0.2	0.55 (0.24 to 1.26), 0.16	
Intermediate vs high		0.71 (0.41 to 1.23), 0.22	0.67 (0.36 to 1.28), 0.23	
Age				
52–65 vs <52		1.53 (0.88 to 2.66), 0.13	1.47 (0.77 to 2.84), 0.25	
66–75 vs <52		1.56 (0.88 to 2.76), 0.13	1.17 (0.59 to 2.36), 0.65	
Re-induction vs induction		1.08 (0.68 to 1.7), 0.75	0.8 (0.45 to 1.43), 0.46	
AA antibiotics (yes vs no)	1.02 (0.56–1.87), 0.94	1.12 (0.64 to 1.96), 0.68	1.39 (0.67 to 2.87), 0.38	0.92 (0.26 to 3.27), 0.9
Risk				
Favourable vs high		0.64 (0.32 to 1.26), 0.19	0.54 (0.24 to 1.24), 0.15	
Intermediate vs high		0.72 (0.41 to 1.24), 0.24	0.65 (0.34 to 1.24), 0.2	
Age				
52–65 vs <52		1.47 (0.85 to 2.54), 0.17	1.47 (0.77 to 2.82), 0.24	
66–75 vs <52		1.49 (0.84 to 2.62), 0.17	1.15 (0.58 to 2.29), 0.68	
Re-induction vs induction		1.06 (0.67 to 1.68), 0.81	0.85 (0.47 to 1.51), 0.57	
CPN3+ (yes vs no)	1.07 (0.61–1.87), 0.82	1.19 (0.7 to 2.03), 0.51	1.49 (0.75 to 2.99), 0.26	0.79 (0.25–2.48), 0.69
Risk			0.56 (0.24 to 1.29), 0.17	
Favourable vs high		0.65 (0.33 to 1.29), 0.22	0.71 (0.37 to 1.35), 0.3	
Intermediate vs high		0.75 (0.43 to 1.3), 0.3		
Age				
52–65 vs <52		1.45 (0.84 to 2.52), 0.18	1.43 (0.74 to 2.76), 0.29	
66–75 vs <52		1.45 (0.82 to 2.57), 0.21	1.1 (0.54 to 2.21), 0.8	
Re-induction vs induction		1.04 (0.66 to 1.64), 0.87	0.8 (0.45 to 1.43), 0.45	
IV vanc (yes vs no)	1.25 (0.69–2.26), 0.46	1.08 (0.65 to 1.79), 0.78	0.74 (0.42 to 1.3), 0.3	4.85 (0.64 to 36.92), 0.13
Risk				
Favourable vs high		0.65 (0.33 to 1.27), 0.21	0.55 (0.24 to 1.26), 0.16	
Intermediate vs high		0.73 (0.42 to 1.26), 0.26	0.67 (0.35 to 1.27), 0.22	
Age				
52–65 vs <52		1.46 (0.84 to 2.52), 0.18	1.5 (0.78 to 2.89), 0.22	
66–75 vs <52		1.49 (0.84 to 2.62), 0.17	1.17 (0.59 to 2.32), 0.65	
Re-induction vs induction		1.03 (0.65 to 1.63), 0.89	0.83 (0.47 to 1.48), 0.53	
Oral vanc (yes vs no)	0.69 (0.28–1.71), 0.42	0.58 (0.25 to 1.35), 0.2	1.02 (0.43 to 2.42), 0.97	0 (0 to Inf), 1
Risk				
Favourable vs high		0.64 (0.33 to 1.27), 0.2	0.55 (0.24 to 1.25), 0.15	
Intermediate vs high		0.72 (0.41 to 1.24), 0.23	0.67 (0.35 to 1.28), 0.23	
Age				
52–65 vs <52		1.51 (0.88 to 2.61), 0.14	1.46 (0.76 to 2.81), 0.26	
66–75 vs <52		1.46 (0.82 to 2.58), 0.19	1.16 (0.58 to 2.31), 0.68	
Re-induction vs induction		1.09 (0.69 to 1.73), 0.7	0.8 (0.45 to 1.43), 0.45	

AA, broad-spectrum anti-anaerobes; AML, acute myeloid leukaemia; CPN3+, third-generation or higher-generation cephalosporins; FQN, fluoroquinolones; IV vanc, intravenous vancomycin; NRM, non-relapse mortality; oral vanc, oral vancomycin; OS, overall survival; RFS, relapse-free survival.

entire course of immunotherapy,[8 23] the most common time frame being less than 2 months before initiation or 1 month after ICI therapy.[7 9 13] Some of the studies also included patients with exposure to antibiotics even several months after initiation of immunotherapy[8 23] which also includes antibiotic exposure at times in which maximal clinical benefit had already been obtained. Nonetheless, a pooled analysis of studies suggested that antibiotic use up to 30 days before ICI initiation has the greatest potential effect on ICI outcomes,[8 14 15] further

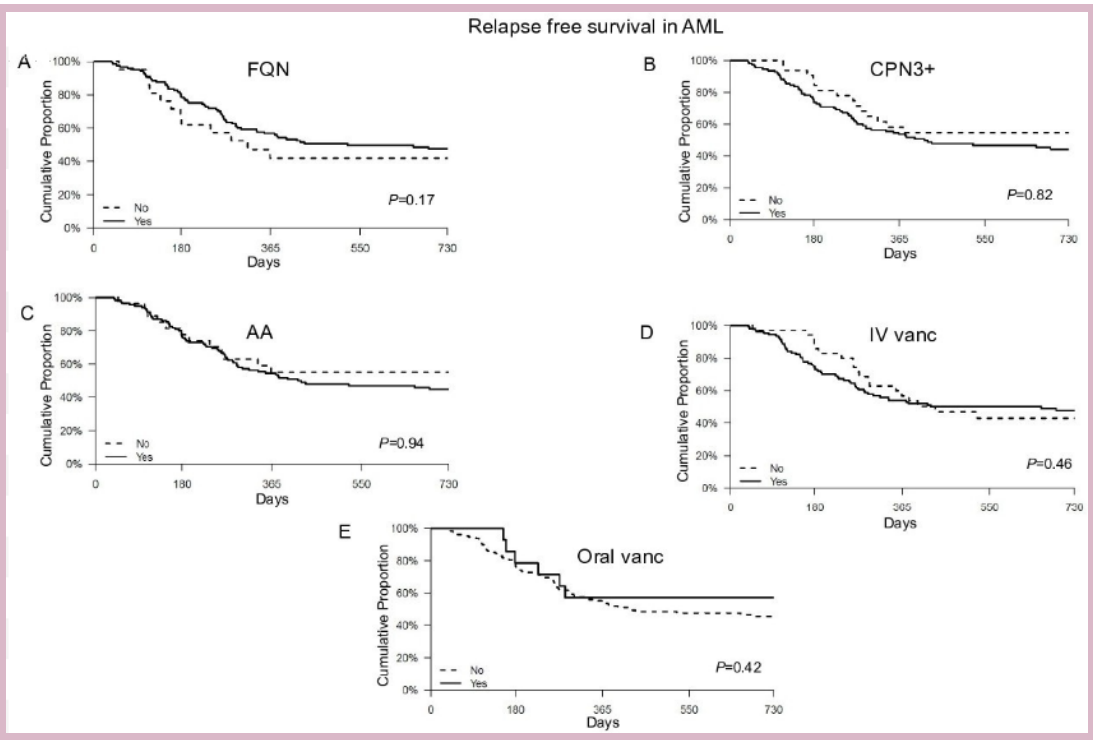

Figure 3 Kaplan-Meier curves of relapse free survival outcomes in acute myeloid leukemia with use of specific antibiotic class based on spectrum of activity. AA, broad-spectrum anti-anaerobic antibiotics; CPN3+, third-generation or higher-generation cephalosporins; FQN, fluroquinolone; IV vanc, intravenous vancomycin; oral vanc, oral vancomycin.

suggesting that stratification by timing of antibiotic exposure is critical.

Unlike the previous studies, which combined all antibiotic exposures as a single event, we tested the effect of antibiotic subgroups depending on the spectrum of antimicrobial activity. In NSCLC, we found significant association of concurrent use of AA antibiotics and 'other' antibiotics on CBR, PFS and OS. Piperacillin-tazobactam was the most commonly used anti-anaerobic and IV vanco was the most commonly used 'other' antibiotics. It has been shown that broad-spectrum antibiotic use may cause sustained impairment of mucosal and systemic immunity by affecting macrophages and cytotoxic T cells.[31 32] Our findings, while exploratory, underscore the importance of distinguishing between concomitant exposure to broad-spectrum combination antibiotic treatments from single-agent or narrow-spectrum antibiotics and sequential exposures to individual antibiotics which may allow enough time for microbiome repopulation between exposures.

Our study in RCC showed a significant association of any antibiotic exposure to PFS, CBR and OS which is in agreement with previous studies.[11 15 22 24] In addition, we showed that use of penicillins, penicillin-class or early-generation cephalosporins was associated with inferior outcomes (CBR and PFS). None of the previous studies looked at the effect of specific antibiotic classes. Notably, cefazolin given as prophylaxis periprocedurally was the most frequently used antibiotic in this group. It can be speculated that the gut microbiome integrity is critical to immunotherapy response in the period leading to immunotherapy and that any derangements even by narrow-spectrum antibiotics can impair its therapeutic efficacy. The difference between the type and pattern of usage of antibiotics between RCC and NSCLC suggests distinctive infection patterns and clinical indication of antibiotics between the two tumour types. An alternative explanation is heterogeneity in the baseline microbiome profile of RCC and NSCLC. The influence of microbiota-induced antitumour immune response may be context-dependent, that is, different species of bacteria may modulate the host immunity depending on the type of cancer. Supporting this argument is that microbiome profiling in preclinical and clinical models of melanoma and NSCLC has shown that bacterial species associated with outcomes were different between studies.[17 19]

Our findings are limited by the retrospective nature of this study and modest sample size. The gut microbiome is dynamic in nature and our study did not assess the relationship between antibiotic use and its immediate and long-term effect on gut microbiome composition. Previously, a study also showed that antibiotic use was associated with reduced rate of immune-mediated diarrhoea or colitis and was also associated with increased risk of severity.[33] In our study, we did not specifically collect data on immune-related toxicities associated with antibiotic exposure in the solid tumour population. Prospective serial assessment of microbiome while on treatment with immunotherapy and following antibiotic exposure should be explored in future studies. Other factors with a potential to affect microbiome include diet, concomitant

medications and probiotics; these factors were not captured in our study. It is possible that our observed associations between antibiotic use and worse survival are not reflective of microbiota–immunity interaction, but due to more antibiotic exposure in more seriously infected patients with higher infection-related mortality. We speculate that this could explain some of our findings in the NSCLC cohort. However, antibiotic use in our RCC cohort was independently associated with worse outcomes after adjustment for other markers of adverse prognosis. Despite these limitations, our findings in RCC are consistent with other studies and further validate the need for systematic investigation of the clinical outcomes of antibiotics stratified by spectrum of antimicrobial activity in the context of immunotherapy. While we know that broad-spectrum antibiotic exposure is more detrimental than narrow-spectrum antibiotics to the microbiome diversity, their differential effect on immunotherapy outcomes is yet to be investigated. There is a need for a consensus on the most critical antibiotic exposure window around ICI and the minimum duration of antibiotic exposure impacting immunotherapy outcomes.

In conclusion, we show that antibiotic use in patients with AML during induction chemotherapy was not associated with RFS. In NSCLC, use of only certain classes of antibiotics were associated with decreased PFS and OS, while for RCC, in general, antibiotic use was associated with inferior outcomes. Use of penicillins, penicillin-class or early-generation cephalosporins in RCC influenced outcomes the most. Validation in other cohorts, mechanistic studies and prospective microbiome profiling are some of the next steps being explored.

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