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ORIGINAL ARTICLE

The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies

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Abstract

Purpose Pre-clinical and early clinical data suggests the microbiome plays an important role in oncogenesis and influences response to immune checkpoint blockade (ICB). The objective of this systematic review and meta-analysis was to determine whether antibiotics affect overall survival (OS) and progression free survival (PFS) in patients with solid malignancies treated with ICB.

Patients and methods A systematic search of EMBASE, MEDLINE and conference proceedings was conducted for observational studies examining the effect of antibiotics on ICB. A random effects study-level meta-analysis was performed with pooling of the hazards ratio (HR) for OS and PFS. Meta-regression was used to determine the impact of the timing of antibiotic exposure on OS.

Results 766 studies were identified, and 18 studies met the inclusion criteria. Of the 2889 patients included, 826 (28.6%) were exposed to antibiotics. The most common malignancies were lung (59%), renal cell carcinoma (RCC) or urothelial carcinoma (16.3%) and melanoma (18.7%). OS was prolonged in those without antibiotic exposure (pooled HR 1.92, 95% CI 1.37–2.68, $p < 0.001$). The effect of antibiotics on OS was greater in studies defining antibiotic exposure as 42 days prior to initiation of ICB (HR 3.43, 95% CI 2.29–5.14, $p < 0.0001$). PFS was also longer in patients who did not receive antibiotics (pooled HR 1.65, 95% CI 1.3–2.1, $p < 0.0001$).

Conclusion In patients receiving ICB, OS and PFS are longer in patients who are not exposed to antibiotics. Antibiotic use in the 42 days before starting ICB appears to be most detrimental to outcome.

Keywords Antibiotics · Immunotherapy · Immune checkpoint blockade · Cancer

Abbreviations

CAR-T	Chimeric antigen receptor T cell
CTLA-4	Cytotoxic T lymphocyte associated protein 4
ECOG	Eastern Co-operative Oncology Group
HR	Hazard ratio
ICB	Immune checkpoint blockade
NSCLC	Non-small cell lung cancer

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OS	Overall survival
PD-1	Programmed cell death protein—1
PD-L1	Programmed death—ligand 1
PFS	Progression free survival
RCC	Renal cell carcinoma

Introduction

There is growing interest in the effects of the microbiome on oncogenesis and response to treatment, particularly ICB. Two landmark studies in mice provided the first evidence that the microbiome may directly impact the effectiveness of immunotherapy [1, 2]. More recently, prospective studies have demonstrated that in patients with metastatic melanoma and non-small cell lung cancer (NSCLC) initiating ICB, responses are in part predicted by microbiome diversity and composition [3–5]. If antibiotics disrupt the ecological balance of the microbiome which is essential for immune activation, exposure to antibiotics may compromise the effectiveness of ICB in routine clinical practice.

Following the discovery that antibiotics had a deleterious impact on ICB activity in pre-clinical models, several institution-based retrospective cohort studies have examined the use of antibiotics in the period immediately preceding or following the initiation of immunotherapy and the impact on clinical outcomes. Some have found shorter OS and PFS in those exposed to antibiotics [6–8]. However, the results have not been uniform, with other studies demonstrating either no difference or survival advantage in those receiving antibiotics [5, 9]. Furthermore, the timing of antibiotic exposure varied considerably.

The primary objective of this systematic review and meta-analysis is to examine the impact of antibiotics on the effectiveness of ICB for solid malignancies, as measured by OS and PFS. The secondary objective is to explore the impact of timing of antibiotic exposure on OS and PFS.

Methods

Study eligibility and identification

A systemic search for cohort studies published in English, examining the association between antibiotic use and ICB using MEDLINE and EMBASE was performed on April 26th, 2019. The search strategy is outlined in Supplemental Fig. 1. The following criteria were required for study inclusion: observational cohort studies, studies addressing the impact of antibiotics on the effectiveness of ICB, adult population, solid tumours, and available HR for PFS and/or OS. Studies looking at Chimeric Antigen Receptor T-cell (CAR-T) therapies and bone-marrow transplant were excluded. All identified articles were reviewed by Wilson and Chin for consensus on inclusion. References of included studies were reviewed for any additional publications by manual search. To limit publication bias, unpublished studies were also included. On June 1st, 2019, abstracts from conference proceedings of the American Society of Clinical Oncology and the European Society of Medical Oncology were searched.

Data extraction

For included studies the following data were extracted: the number of patients, type(s) of malignancy, study type (retrospective vs prospective), number of patients exposed to antibiotics, definition of antibiotic exposure, type of ICB agent (anti-PD1/PDL1, anti-CTLA-4, or combination treatment), the proportion of patients with ECOG 0-1, median age and proportion of male patients. Median OS and median PFS, associated HR and 95% Confidence Intervals (CI) for those exposed and not exposed to antibiotics were obtained for each included study. All median OS and PFS times were converted to months (weeks multiplied by 7 divided by 30 to calculate the duration in months). Where the HRs were not available in the presented data, they were derived from the Kaplan-Meir curves using the methodology by Tierney et al. [10]. If the required data was not immediately available from the published abstracts or papers, authors were contacted directly for results.

Statistical analyses

Given the heterogeneity of the studies and patient population, a random effects model was used to pool estimates of effect size for OS and PFS. The χ^2 Cochrane Q test was used to detect heterogeneity across the different studies. The definition of antibiotic exposure was categorised into three groups: group 1 publications defined the window of antibiotic exposure as 42 days prior to ICB until initiation at time 0; group 2 publications defined antibiotic exposure as 60 days before and up to 42 days after initiation of ICB; and group 3 publications defined antibiotic exposure as 60 days before and anytime during ICB treatment. The pooled OS and PFS were stratified by antibiotic exposure definition, and we used a test for heterogeneity to determine whether differences between the groups were significant. Meta-regression was also used to examine the effect of antibiotic exposure timing on OS. The HR for OS and PFS were also stratified by type of malignancy to test for heterogeneity. Publication bias was evaluated by examining the funnel plot of the effect size for each observational study against the reciprocal of its standard error. The nominal level of significance was predetermined to be 5% with the exception of publication bias, where significance for the Egger's test was predetermined to be 10%. All 95% confidence intervals were two-sided.

Results

Studies for inclusion

A total of 751 articles were identified by systematic search, and an additional 15 were identified by manual search of references and conference abstracts. After removal of duplicates and review of the title and/or abstract, 33 studies remained (Fig. 1). A further four abstracts were duplicates of included publications, seven studies were excluded as no univariate HR for OS or PFS were available even after contacting the authors and two were excluded as they were not observational studies [11, 12]. One study [9] was excluded as the population of patients was re-analysed in the subsequent publication by Routy et al. [13]. Another study [14] was excluded as the patients were duplicated in Huemer et al. [5]. The RCC cohort in Routy et al. [13] was excluded as it was updated in the paper by Derosa et al. [7] (Supplemental Table 1). Four studies [5, 7, 13, 15] included separate cohorts and these are presented individually in this meta-analysis. Therefore, a total of 22 cohorts are presented from this point forward for convenience.

All included studies were published between 2017 and 2019. The studies were conducted predominantly in North America and Europe. One study was prospective [16], while all other studies were retrospective.

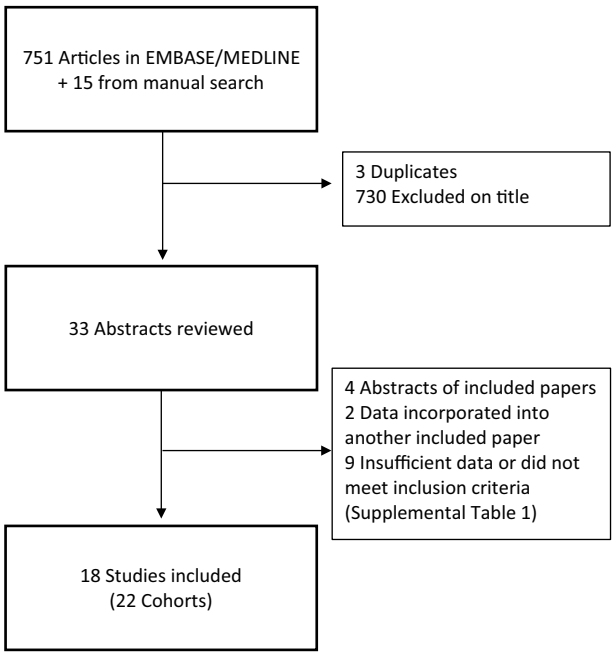

Fig. 1 Search strategy

Baseline data

A total of 2889 patients were included in this meta-analysis and 826 (28.6%) were exposed to antibiotics. The most common types of malignancy were lung (59%), renal cell carcinoma or urothelial carcinoma (16.3%) and melanoma (18.7%). There were more men included (63.7% of 2408 patients for whom sex was reported). The class of immune-checkpoint blockade was clearly documented for 90% of patients included. The majority of these patients were treated with programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) inhibitors (92.8%), 5.2% with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors and 1.7% combination PD1/PDL1 plus CTLA-4 inhibitor. Functional status was reported in 49.5% of patients, and 84% were ECOG 0-1 (Table 1).

The definition of antibiotic exposure varied considerably across the included cohorts (Fig. 2). Some cohorts defined antibiotic exposure as treatment anytime during ICB [15, 17, 18, 21], while others included narrow definitions such as antibiotic exposure 14 days before or after ICB initiation [6].

Outcome data

OS data was available for 21 of the 22 cohorts included. Pooled results showed a prolonged OS among those who did not receive antibiotics (pooled HR 1.92, 95% CI 1.37–2.68, $p < 0.001$) (Fig. 3). There was heterogeneity in the results for OS between studies (Cochrane Q test for heterogeneity $p < 0.0001$). The funnel plot showed no publication bias for the OS data, with Egger test for small studies $p = 0.397$ (Supplemental Fig. 2).

The HR for OS were stratified by the cohort definition of antibiotic exposure (Fig. 3). Among group 1 cohorts (antibiotic exposure within 42 days before initiation of ICB), OS was prolonged in those who were not exposed to antibiotics (HR 3.43, 95% CI 2.29–5.14, $p < 0.0001$). For group 2 cohorts (antibiotic use within 60 days before or 42 days after initiation of ICB) those unexposed to antibiotics still had prolonged OS, but the effect was less pronounced (HR 1.81, 95% CI 1.29–2.54, $p = 0.001$). However, for group 3 cohorts (antibiotic exposure 60 days before and anytime during ICB) there was no difference in OS between those exposed and unexposed to antibiotics (HR 0.89, 95% CI 0.42–1.90, $p = 0.76$). Meta-regression demonstrated a strong association between the antibiotic window and the effect of antibiotics on OS ($p = 0.002$, Supplemental Fig. 3). Overall, these results suggest that the impact of antibiotics on OS is greatest in the period immediately prior to initiation of immunotherapy. When the HRs for OS were stratified by tumour type, we also found differences in the effects of antibiotics on outcome (NSCLC HR 2.00 95% CI 1.23–3.25, RCC HR 1.86 95% CI 1.16–2.98, melanoma HR 1.08 95% CI

Table 1 Characteristics of included studies

Study	Study type	*N*	Antibiotic exposed *N* (%)	Cancer type	Immunotherapy target (*N*)	Median age (Abx vs no Abx) or pooled median	ECOG 0–1 or KPS ≥ 90 *N* (%)	Male *N* (%)	Median PFS Abx vs no Abx (months)	Median OS Abx vs no Abx (months)
Group 1 cohorts										
Derosa-RCC [7] (France/USA)	Paper	121	16 (13%)	RCC	PDL1 (111) PDL1 + CTLA-4 (10)	61 vs 61		118 (49%)	1.9 vs 7.4	17.3 vs 30.6
Derosa-NSCLC [7] (France/USA)	Paper	239	48 (20%)	NSCLC	PDL1 (205) PDL1 + CTLA-4 (34)	63 vs 66	236 (99%)	80 (66%)	1.9 vs 3.8	7.9 vs 24.6
Elkrief [8] (Canada)	Paper	74	10 (13.5%)	Melanoma	PD1/PDL1 (54) CTLA-4 (20)	58 vs 65		49 (66%)	2.4 vs 7.3	10.7 vs 18.3
Pinato [16] (UK)	Abstract	196	97 (49%), only 29 in 0–30 days	NSCLC (119) Melanoma (38) Other (39)	PD1/PDL1 (189) Not specified (7)		159 (84%)	137 (70%)		2 vs 26
Thompson [22] (USA)	Abstract	74	18 (24%)	NSCLC	PD1/PDL1 (74)	66		41 (55%)	2.0 vs 3.8	4.0 vs 12.6
Sen [19] (USA)	Paper	172	19 (11%)	NSCLC 2(1) RCC (25) Melanoma (16) Other (110)	CTLA-4 (105) PDL1 (67)	60		88 (51%)	2.5 vs 3.0	4.6 vs 8.2
Zhao [23] (China)	Paper	109	20 (18.3%)	NSCLC	PD1 (109)	57 vs 62	107 (98%)	89 (82%)	3.7 vs 9.6	6.1 vs 21.9
Group 2 Cohorts										
Ahmed [6] (USA)	Paper	60	17 (28%)	Lung (34) Melanoma (3) RCC (4) Other (19)	PD1/PDL1 (60)	52 vs 66	38 (63%)	35 (58%)	5.0 vs 12.0	5.6 vs 20.8
Hakozaki [41] (Japan)	Paper	90	13 (14%)	NSCLC	PD1 (90)	67 vs 68	77 (85%)	57 (63%)	1.2 vs 4.4	8.8 vs NR
Huemer-Salzburg [5] (Austria)	Paper	43	20 (46.5%)	NSCLC	PD1/PDL1 (43)	60	30 (70%)	21 (49%)		7.5 vs 13.6
Huemer -Linz [5] (Austria)	Paper	53	18 (33.9%)	NSCLC	PD1/PDL1 (53)	66	53 (100%)	32 (60%)		NR vs 10.8
Lalani [26, 42] (USA)	Abstract	146	31 (21%)	RCC	PD1/PDL1 (146)	61		104 (71%)	2.6 vs 8.1	65% vs 79% (1-year OS)
Mielgo-Rubio [20] (Spain)	Abstract	168	80 (48%)	NSCLC	PD1/PDL1 (168)	65	121 (72%)	79.8%	5.1 vs 7.3	8.1 vs 11.9
Routy-NSCLC [13] (France)	Paper	140	37 (26%)	NSCLC	PD1/PDL1 (140)	64	129 (92%)	102 (73%)	2.8 vs 3.5	8.3 vs 15.3

Table 1 (continued)

Study	Study type	*N*	Antibiotic exposed *N* (%)	Cancer type	Immunotherapy target (*N*)	Median age (Abx vs no Abx) or pooled median	ECOG 0–1 or KPS ≥ 90 *N* (%)	Male *N* (%)	Median PFS Abx vs no Abx (months)	Median OS Abx vs no Abx (months)
Routy-Urothelial [13] (France)	Paper	42	12 (29%)	Urothelial	PD1/PDL1 (42)	63	26 (62%)	30 (71%)	1.8 vs 4.3	11.5 vs NR
Schett [24] (Switzerland)	Abstract	218	Estimated at 42 (20%) from power calculation	NSCLC	PD1/PDL1 (218)				1.4 vs 5.8	10.6 vs 29.9
Tinsley [25] (UK)	Paper	291	92 (32%)	Melanoma (179) NSCLC (64) RCC (48)		66	230 (79%)	181 (62%)	3.1 vs 6.3	10.7 vs 21.7
Group 3 Cohorts										
Do [17] (USA)	Abstract	109	87 (80%)	Lung cancer	PD1 (109)					5.4 vs 17.2
Hemadri [21] (USA)	Abstract	172	29 (17%)	Melanoma	PD1/PDL1 (172)			106 (32%)	16.6 vs 19.8	23.8 vs 35.4
Kulkarni-NSCLC [15] (USA)	Abstract	148	87 (59%)	NSCLC	PD1/PDL1 (148)				5 vs 2.5	13 vs 8
Kulkarni-RCC [15] (USA)	Abstract	55	40 (72%)	RCC	PD1/PDL1 (55)				2.9 vs 5	
Masini [18] (Italy)	Abstract	169	59 (35%)	NSCLC (78) Melanoma (57) RCC (29) Other (5)	PD1/PDL1 (159) CTLA-4 (10)					

Abx antibiotic, *NR* not reached, *KPS* Karnofsky Performance Score

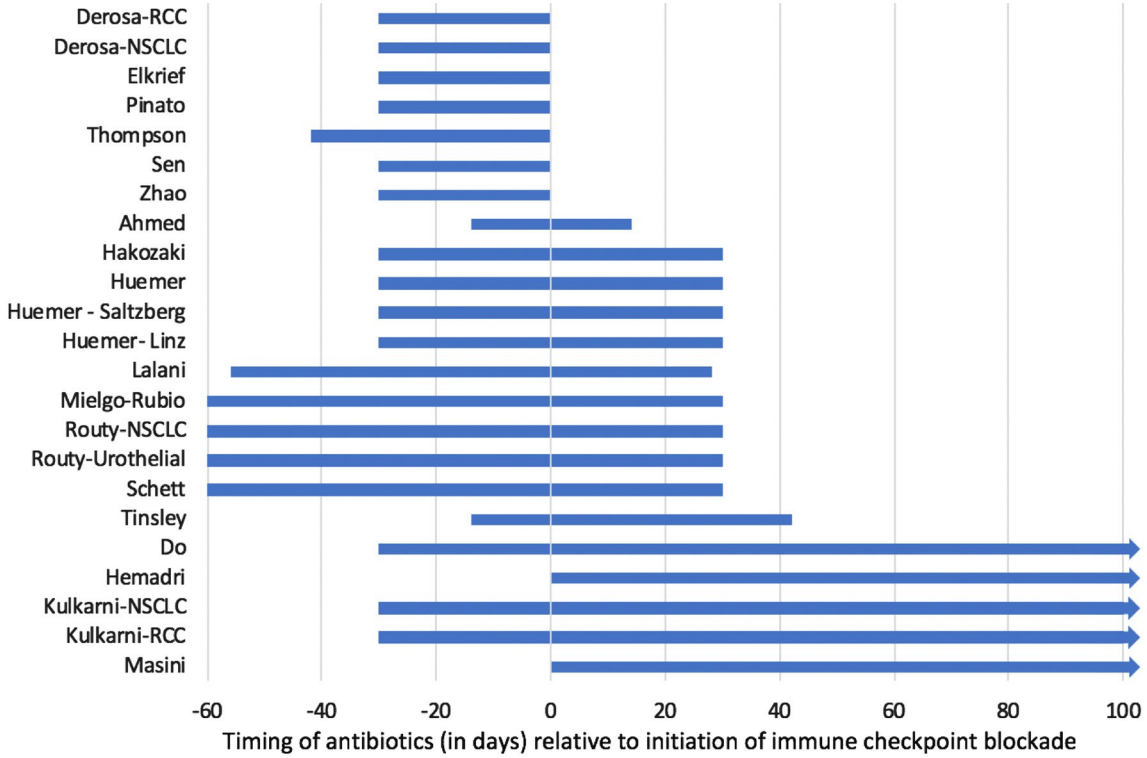

Fig. 2 Definitions of antibiotic exposure by study relative to the initiation of immune checkpoint blockade at time 0. This figure demonstrates the various definitions of antibiotic exposure adopted in each study, relative to the initiation of ICB at time 0. Studies by Do [17], Kulkarni [15], Masini [18] and Hemadri [21] defined antibiotic exposure as any time during ICB, as illustrated with the arrow beyond 100 days

0.4–2.92, mixed tumour types HR 2.07 95% CI 0.94–4.57, test for heterogeneity between subgroups $p = 0.06$) (Supplemental Fig. 4).

PFS data was available for 16 of the 22 cohorts included. Pooled PFS was longer in patients who did not receive antibiotics compared to those who were treated with antibiotics (pooled HR 1.65, 95% CI 1.3–2.1, $p < 0.0001$) (Fig. 4). There was significant heterogeneity between cohorts, and between the groups of cohorts when stratified by antibiotic exposure window (test for heterogeneity between groups $p < 0.001$). The effect of antibiotics on PFS was greatest in group 1 cohorts (HR 2.1 95% CI 1.44–3.06) followed by group 2 cohorts (HR 1.66 95% CI 1.4–1.96). There was no effect of antibiotics on PFS in group 3 cohorts (HR 0.88 95% CI 0.42–1.86). There was no evidence of publication bias in the funnel plot, confirmed by the Egger test for small study effect ($p = 0.12$) (Supplemental Fig. 5). PFS was longer in patients who did not receive antibiotics in cohorts of NSCLC patients (HR 1.64 95% CI 1.07–2.52) and RCC (HR 2.13 95% CI 1.54–2.93) and mixed tumour types (HR 1.47 95% CI 1.16–1.86), but not for melanoma (HR 1.54 95% CI 0.33–7.12) (Supplemental Fig. 6).

Of the included cohorts, 7 [5, 17–21] did not present any multivariate analysis (Supplemental Table 2). Of the 12 cohorts that reported a multivariate analysis for OS, 8 [6, 7, 13, 16, 22–25] remained significant after adjusting for other variables. Of the ten cohorts that presented a multivariate analysis for PFS, 9 [7, 8, 13, 15, 22, 23, 25, 26] remained significant after adjusting for other variables. The types of variables included in the multivariate models differed by cohort as listed in Supplemental Table 2. These results show that even after adjusting for baseline confounders, antibiotics remained associated with worse OS in 66.7% of cohorts and worse PFS in 90% of cohorts for which adjusted analysis was performed.

Discussion

Our pooled results demonstrate that OS was almost two times longer and PFS was 1.65 times longer in patients who did not receive antibiotics either before or during treatment with ICB.

This is the first meta-analysis to characterise how timing of antibiotic exposure might impact on responsiveness to ICB. OS was 3.4 times longer in patients who did not receive any antibiotics in the 42 days prior to ICB (group 1 cohorts). In contrast, pooled results from studies defining antibiotic

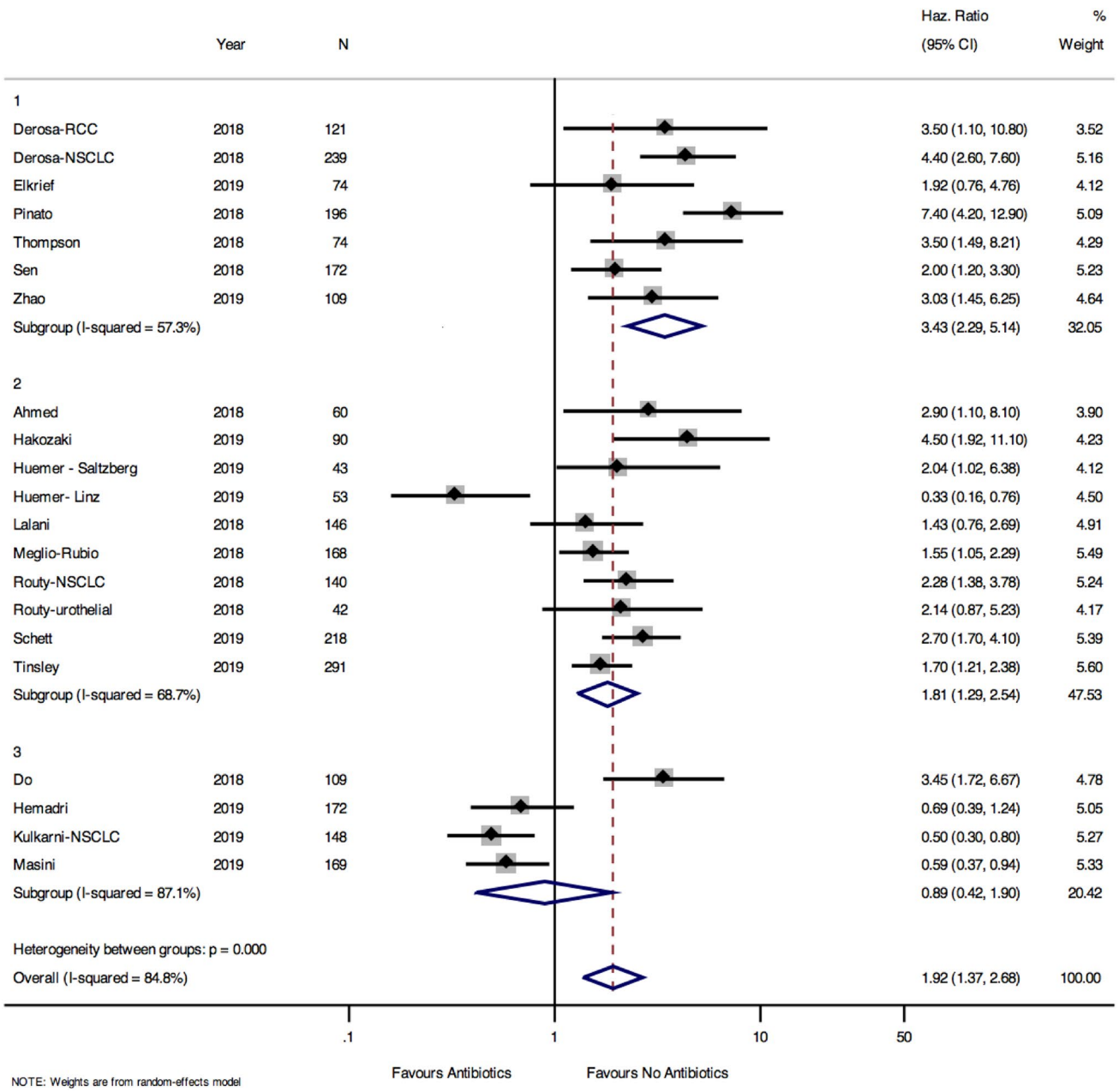

Fig. 3 Pooled hazards ratio for overall survival among those exposed and unexposed to antibiotics stratified by antibiotic exposure definition. Group 1: cohorts defining antibiotic exposure as up to 42 days before initiation of ICB. Group 2: cohorts defining antibiotic exposure as 60 days before and 42 days after initiation of ICB. Group 3: cohorts defining antibiotic exposure as 60 days before and anytime during ICB

exposure as 60 days prior or any time during ICB found no differences in OS (group 3 cohorts). These pooled findings are also supported by stratified analyses within studies. Sen et al. [19] presented a stratified analysis by timing of antibiotic onset, and found no significant difference in overall survival in patients receiving antibiotics during ICB or in the 30–60 days prior to ICB initiation, as opposed to the significant difference in OS for antibiotic use 0–30 days prior to IO initiation. Pinato et al. [27] also stratified antibiotic exposure by timing, and found that the effects of antibiotics on survival were much greater in those exposed 0–30 days before initiation of immunotherapy (HR 7.4, 95% CI 4.3–12.8), compared to those exposed during immunotherapy (HR 0.9, 95% CI 0.5–1.4). In a recent study [28] of 12 healthy men treated with a 4-day course of meropenem, vancomycin and gentamicin, the gut microbiota composition had recovered to near baseline by 42 days. Another study treated three patients with ciprofloxacin and found that most species had

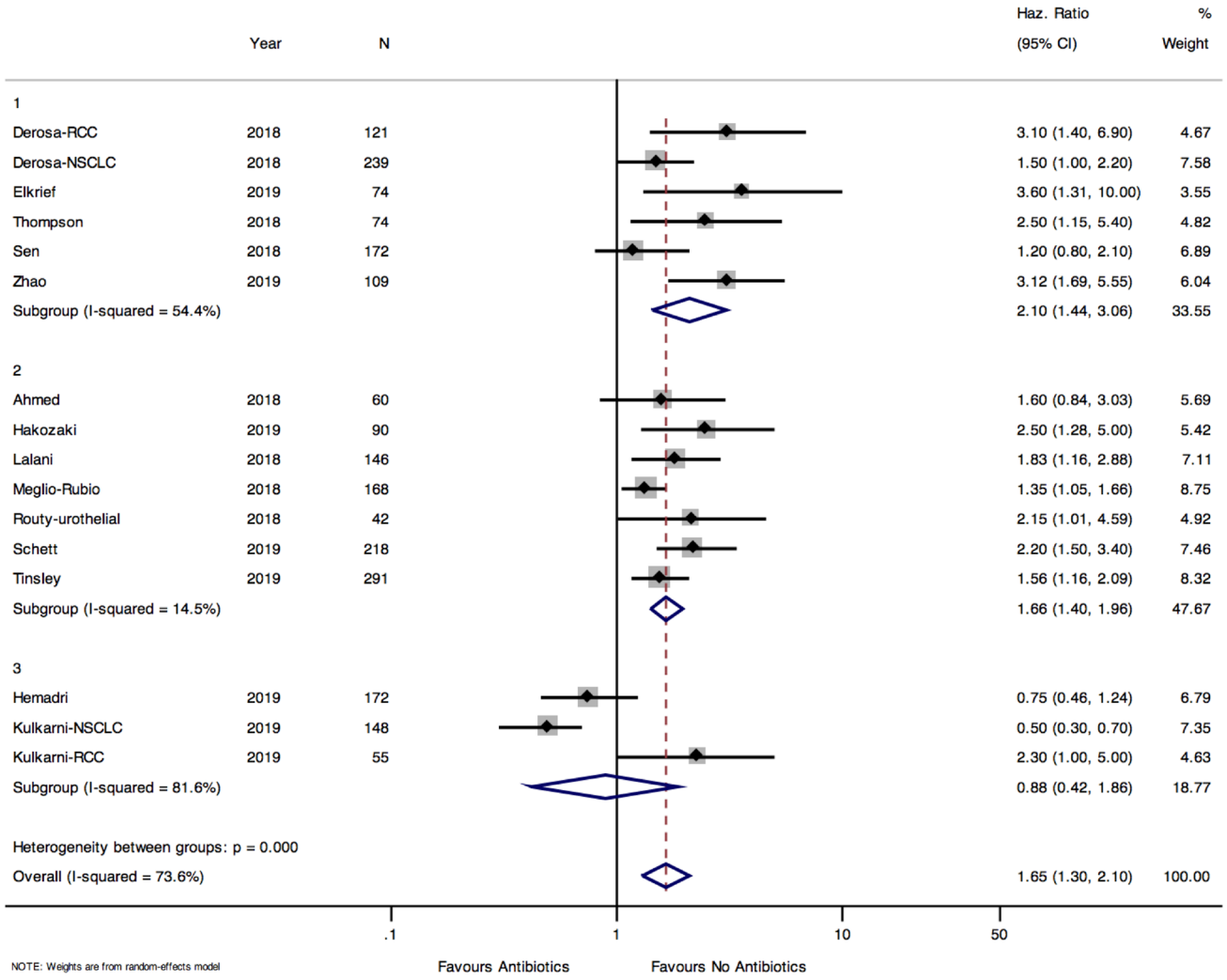

Fig. 4 Pooled hazards ratio for progression free survival among those exposed and unexposed to antibiotics stratified by antibiotic exposure definition. Group 1: cohorts defining antibiotic exposure as 42 days before initiation of ICB. Group 2: cohorts defining antibiotic exposure as 60 days before and 42 days after initiation of ICB. Group 3: cohorts defining antibiotic exposure as 60 days before and anytime during ICB

recovered to a pre-antibiotic state by 4 weeks [29]. These recovery times support our findings that antibiotic exposure in the 42-day period prior to start of immunotherapy is particularly harmful to the microbiome, and most likely to negatively impact on the effectiveness of ICB.

While there is evidence to support the critical window prior to initiation of ICB, our understanding of the critical window after initiation of ICB is limited. Pre-clinical models to explore how long the microbiome takes to prime and activate the immune system after exposure to ICB are needed.

The antibiotic exposure windows defined in our study are broad and overlapping. For example, the exposure window in group 3 cohorts (60 days prior and any time during ICB) also includes patients exposed to antibiotics within the definition of group 1 cohorts (42 days prior to ICB initiation). This may explain some of the heterogeneity seen within the results for group 2 and 3 cohorts, as opposed to group 1 cohorts, where the results are more homogenous. Moreover, we cannot be certain that a patient exposed to antibiotics within 42 days of initiation of ICB, was not also later exposed to antibiotics during treatment with ICB. Unfortunately, detailed data regarding the timing of exposure for each patient was not available in the setting of this study-level meta-analysis, and therefore, this result should be considered hypothesis generating. A patient-level analysis to more precisely explore the timing of antibiotic exposure is needed.

When the pooled HR for OS were stratified by tumour type, overall survival was prolonged in patients with NSCLC and RCC/urothelial cancers who were not exposed to antibiotics. While we recognise that RCC and urothelial cancers have different biology and responses to immunotherapy, the

effect of antibiotics on outcome in the RCC and urothelial cancer studies was similar, as demonstrated by the similar HR for PFS and OS between these studies. Antibiotic exposure did not affect OS in the studies of melanoma patients. This likely reflects the small number of melanoma studies included in the meta-analysis (only 2 cohorts). This result may also be confounded by the broad definition of antibiotic use adopted in one [21] of these two studies, or by other baseline differences between the populations.

Metagenomic research is ongoing to further clarify which bacterial species predict response to ICB. Several studies have demonstrated differences in the stool microbial composition in responders and non-responders to ICB for lung, renal [13] and melanoma [3, 4, 30, 31] patients. However, the bacterial signatures correlating with response to ICB have not yet been validated in a prospective trial. The bacteria that predicted response to therapy differed between studies. Frankel et al. found that the presence of *Bacteroides*, among others, predicted ICB response, while Chaput found that *Faecalibacterium* was predictive of response. Matson et al. identified *Bifidobacterium longum* as a marker of ICB response, while Gopalakrishnan et al. identified *Ruminococcaceae*. Finally, Routy et al. found a correlation between the abundance of *Akkermansia muciniphilia* in stools and clinical response to ICB. Different microbial species have diverse immunomodulatory effects independent of microbial phylogeny [32], supporting the hypothesis that imbalances in the gut flora might alter the immune systems' ability to respond to ICB for malignancy, rather than the presence or absence of any one particular species. Additionally, pre-clinical models have shown that poor response to ICB may be reversed by faecal compensation with bacteria [13, 30], raising the possibility of improving responsiveness to ICB through therapeutic manipulation of the microbiome. A recent randomised study of oral supplementation of *Akkermansia muciniphilia* improved several metabolic parameters in obese patients, providing a proof of concept that oral manipulation of the microbiome could be used to alter disease outcomes [33], and similar studies in cancer are needed. The mechanisms by which the microbiome primes or activate the immune system's response to ICB remains an area of active research.

To date, no circulating markers of gut health have been prospectively validated to predict response to immunotherapy. However, in a recent study, patients responding to nivolumab for NSCLC were found to have higher plasma citrulline levels compared to non-responders [34]. Citrulline is an amino acid produced almost exclusively by enterocytes. It has been validated as a marker for chemotherapy induced mucosal barrier injury in paediatric patients [35]. Its role in predicting gut health in the setting of antibiotic use and response to immunotherapy should be further explored. Moreover, this same study [34] examined the blood microbiome and found several signatures that were predictive of clinical response to ICB. Whether or not antibiotics impact on the blood microbiome and negatively affect PFS and OS remains to be seen.

The microbiome may also play a role in modulating immune related toxicities. Vetizou et al. [1] demonstrated that oral inoculation with *Bacteroides* in combination with *Burkholderia* reduced immune related colitis in mice. Therefore, antibiotic use could affect the severity and frequency of immune related toxicities in patients treated with ICB. In one retrospective study [14], there was no difference in the grade of immune related toxicities in patients exposed or unexposed to antibiotics. However, further research is warranted.

There are several important limitations of this study. This systematic review and meta-analysis only included observational studies. However, our search identified two studies [11, 12] analysing prospective randomised controlled trial data. In a pooled analysis of the OAK [36] and POPLAR [37] trials for NSCLC, median OS was shorter among those exposed to antibiotics (8.54 vs 14.06 months, HR 1.32, 95% CI 1.06–1.63), and this association remained significant after adjusting for potential confounders [12]. On the other hand, Weinstock et al. [11] pooled results from seven clinical trials in urothelial cancer and found no difference in OS (9.23 vs 9.86 months) or PFS (105 vs 101 days). For the latter study, antibiotic exposure was defined as any time during ICB, further supporting our results that the timing of antibiotic exposure is important. Therefore, while these studies are not included in the pooled results of this meta-analysis, they provide further support for the findings presented in this paper. Similar analyses for other large randomised studies of immunotherapy in solid malignancies would be helpful, with attention paid to the antibiotic exposure window.

Secondly, this study does not account for differences in the types of antibiotics, route of administration or duration of use. Longer treatment durations and broader spectrum antibiotics may have more detrimental effects on gut microbiome, thereby having a greater impact on OS and PFS. Ahmed et al. [6] found PFS was longer in patients receiving narrow vs broad spectrum antibiotics; however, the sample size was small (HR 1.8, 95% CI 0.86–3.89). Tinsley et al. [25, 38] found that longer durations and multiple courses of antibiotics had more significant effects on OS. Galli et al. [39] also found that patients with longer antibiotic exposures had shorter PFS and OS. Mielgo-Rubio found that patients who received intravenous antibiotics has shorter OS and PFS compared to those who received oral antibiotics (OS 2.9 vs 14.2 months, $p = 0.0001$, PFS 2.2 vs 5.9 months, $p = 0.001$) [20]. Unfortunately, there was insufficient data to examine the effect of antibiotic type, duration and route on clinical outcomes in this study and further research is warranted. Furthermore, our study was not able to determine whether the

detrimental effects seen on OS and PFS from antibiotic exposure are due to the antibiotics themselves, or whether having an infection alone might negatively influence the response to immunotherapy. Further studies examining the PFS and OS stratified by antibiotic indication (prophylactic antibiotic use vs treatment of infection) could help to clarify this important point.

Thirdly, this study-level meta-analysis cannot adjust for patient-level confounders. Differences in baseline performance status, age and comorbidities among those treated with antibiotics could drive poorer survival outcomes and cannot be accounted for in the present study. Among the cohorts that did present a multivariate analysis, antibiotics remained associated with worse OS in 66.7% and worse PFS in 90%. Further research adjusting for potential confounders is needed.

Finally, while this study focused on the impact of antibiotic exposure on OS and PFS, there is growing literature that proton-pump inhibitors, corticosteroids and vaccines may also influence the outcomes and safety of ICB [40]. Further exploration of how these drugs might impact the microbiome thereby indirectly impacting response to ICB should be undertaken.

Conclusions

In this meta-analysis of 18 observational studies including 22 distinct cohorts, pooled HR for OS and PFS were longer in patients who were not exposed to antibiotics. The timing of antibiotic exposure is a significant effect modifier in the association between antibiotics and response to ICB, with antibiotic exposure immediately prior to initiation of ICB having the greatest impact on OS. Without undermining the role of antibiotics in patients with infections, careful consideration of the use of antibiotics and the subsequent timing of ICB initiation is warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval As this study was based on published data, no ethics approval was sought for the study.

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