Supplementary Online Content

Pinato DJ, Howlett S, Ottaviani D, et al. Association of prior antibiotic treatment with survival and response to immune checkpoint inhibitor therapy in patients with cancer. Published online September 12, 2019. *JAMA Oncol*. doi:10.1001/jamaoncol.2019.2785

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

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eMethods.

Patients.

Patients with a diagnosis of advanced malignancy undergoing treatment with immune checkpoint inhibitors (ICPI) for an approved oncological indication were identified from Oncology Pharmacy electronic records and entered into a prospectively maintained database. At the censoring date of the 15th of August 2018, this database included a total of 196 patients treated within the Departments of Oncology at Imperial College NHS Trust and Chelsea and Westminster NHS Foundation Trust between year 2015 and 2018. Clinico-pathologic variables including overall survival (OS) were derived from electronic medical records. OS was calculated from the date of ICPI commencement until last follow up or patients' death. Response to ICPI was evaluated according to RECIST criteria (version 1.1) and best responses to ICPI were recorded for each evaluable patient. Electronic medical records were reviewed to identify prescription of oral or intravenous antibiotic therapy (ATB). ATB was defined on the basis of timing of administration as pre-ICPI ATB (pATB) if received during a 30-days interval prior to ICPI initiation or concurrently with ICPI therapy (cATB) if prescribed from the first ICPI dose until cessation. We elected to use a 30-days cut-off point from antibiotic exposure to ICPI initiation on the basis of longitudinal data on bacterial diversity demonstrating that the compositional changes in the gut microflora tend to completely recover by 1 month after antibiotic dosing[1]. The choice of a 30-days interval is in line with subsequent evidence in cancer patients receiving ICPI where prognostic and disease-modulating effect from antibiotic exposure were maximal at 30 days[2]. We recorded and presented corticosteroids use following pre-specified methodology published by Arbour et al. and evaluated patients who received $\geq$ 10 mg of prednisone equivalent at the time and during ICPI treatment[3].

Ethical approval to conduct this study was granted by the Imperial College Tissue Bank (Reference Number R16008) under a broader protocol to investigate tissue and clinical predictors of outcome in patients with

metastatic malignancies undergoing ICPI treatment. All study related procedures and data collection were conducted in accordance to the Declaration of Helsinki and in accordance with Good Clinical Practice.

Statistical analysis.

We presented patient characteristics as means or medians as appropriate, and used Pearson chi-square or Fisher's exact tests for analysis of proportions across groups. We plotted group-specific OS using the Kaplan-Meier method and tested for between-group difference using the Log-rank test. We performed uni- and multivariable survival analyses of survival using Cox regression models to evaluate the impact of pATB on patient OS. Due to the possibility of reduced power related to missing covariable data, the multivariable model included only the two covariables associated with OS: pATB and best radiologic response.

We supplemented standard multivariable analyses using propensity score modelling. We implemented inverse-probability propensity-score weighting due to better performance in simulation studies with Cox models[4] and used stabilised inverse-probability weights to minimise weight inflation for individuals with low propensity score[5]. The propensity score model included the following variables potentially associated with antibiotic therapy: pre-treatment corticosteroids, tumour type, stage, performance status, and age.

All statistical analyses were performed using SPSS version 21.0 (IBM Inc., Chicago, IL, USA), GraphPad PRISM (GraphPad software inc., La Jolla, CA, USA) and R 3.4.4, with all estimates being reported with corresponding 95% confidence intervals and a two-tailed level of significance of $p<0.05$.

eTable 1. Supplementary clinical characteristics of patients receiving ICPI.

Baseline characteristic	n=196, (%)
Primary site	
Non-small cell lung cancer	118 (60)
Malignant Melanoma	38 (20)
Head & Neck Carcinoma	10 (5)
Renal Cell Carcinoma	11 (6)
Urothelial Carcinoma	16 (8)
Other sites	2 (1)
Albumin (g/L)	34
Median (range)	(20-43)
White Cell Count (cells/cm3)	7.8
Median (range)	(4.1-35.0)
Hb (g/L)	12.1
Median (range)	(7.9-15.4)
Stage at ICPI commencement	
Loco-regional lymph node spread	31 (16)
Metastatic disease	165 (84)
Number of prior lines of treatment	
0	120 (62)
$\geq$1	76 (38)
Treatment modality prior to ICPI	
Surgery	71 (36)
Radical Radiotherapy	38 (20)
Palliative Radiotherapy	65 (33)
Chemotherapy	115 (60)
Targeted therapy	20 (10)
ICPI treatment	
Anti-PD(L)-1 monotherapy	18 (27)
Anti-PD-1/CTLA-4 combination therapy	7 (11)

eTable 2. Indications and characteristics of antibiotic therapy in patients receiving ICPI.

		pATB (=29)	cATB (n=68)
ATB Class	β-lactam ± inhibitors (± other)	20	46
	Quinolones (± other)	5	7
	Macrolides	1	7
	Sulfonamides	1	0
	Tetracyclines	2	5
	Aminoglycosides	1	3
	Nitromidazole	1	3
Duration of ATB treatment	≤7 days / >7 days	26/3	39/29
Number of ATB courses	1 / >1	29/0	44/24
Indication for ATB treatment	Respiratory Tract Infection	17	40
	Urinary Tract Infection	5	3
	Gastrointestinal Infection	1	5
	Skin/Soft tissue infection	2	10
	ENT infection	1	1
	Unclear source	3	15

eTable 3. Uni- and multi-variable analyses of survival in patients treated with ICPI (n=196).

Associations reaching statistical significance (p<0.05) are marked with an asterisk (*).

** For the purpose of survival analysis patients who received no corticosteroid treatment (n=145) and doses of corticosteroids < 10 mg of prednisone equivalent (n=5) were categorised In the same group.

Characteristic		Univariable Analysis		Multivariable Analysis	
		HR (95%CI)	p-value	HR (95%CI)	p-value
Tumour Group, NSCLC MM Others	119/38/39	- 1.33 (0.7-2.4) 1.71 (0.9-3.0)	 0.17 0.33	-	-
ECOG PS, 0/1/2/3	67/92/26/4	1.7 (1.3-2.4)	**<0.001***	-	-
Stage Loco-regional/Metastatic	31/165	1.3 (0.6-2.8)	0.36	-	-
Tumour Burden, <2 metastases/ >2metastases	141/52	2.1 (1.3-3.4)	**0.002***	-	-
Age, <65/>65	84/111	0.9 (0.6-1.5)	0.90	-	-
Corticosteroids use** No/Yes	150/45	1.1 (0.6-1.3)	0.63	-	**-**
Response to ICPI CR/PR/SD/PD	2/65/23/87	8.4 (4.3-16.2)	**<0.001***	8.2 (4.0-16.0)	**<0.001***
pATB No/Yes	167/29	7.4 (4.3-12.8)	**<0.001***	3.5 (1.9-6.1)	**<0.001***
cATB No/Yes	128/68	0.9 (0.5-1.4)	0.65	-	-

eTable 4. The relationship between prior antibiotic treatment (pATB) and the incidence of immune-related adverse events (irAE) in patients who died whilst on ICPI treatment (n=18).

	pATB-	**pATB+**
No irAE	9	5
irAE G1-2	2	1
irAE G3-4	1	0
Total	12	6
Chi-Square p	0.641	

eTable 5. The relationship between survival and response to ICPI and class of antibiotic received prior to immunotherapy.

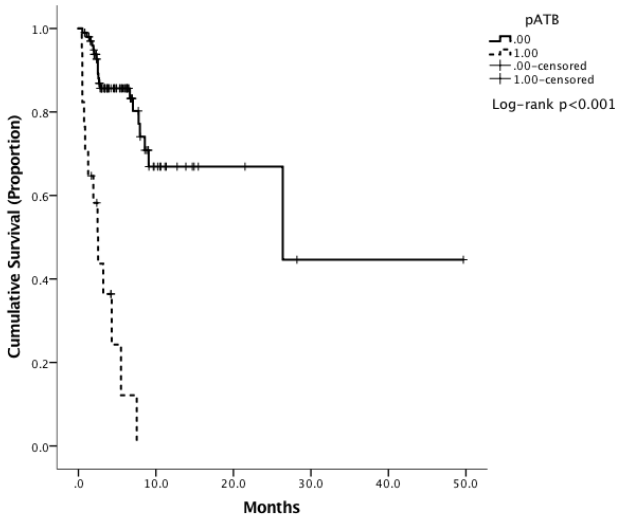
Antibiotic Class	**N**	**Median OS (95%CI)**	**ATB exposure within 30 days from ICPI**	**ICPI response (primary progression/objective response)**	**P value for response (Fisher Exact Test)**
Beta-lactams	22	2.5 (0.5-4.4)	Beta-lactams only (20) Beta lactams + macrolide + carbapenem (1) Beta lactams + sulphonamides (1) Beta lactams + nitrofurantoin (1)	14/5	0.27
Other class	7	1.9 (1.1-2.7)	Quinolones only (3) Quinolones + nitrofurantoin (1) Quinolones + aminoglycoside (1) Tetracyclines only (2)	7/0	

eTable 6. Multivariable Cox regression using propensity score weights. The propensity score model included the following variables potentially associated with antibiotic therapy: corticosteroids use, tumour type, stage, performance status, and age.

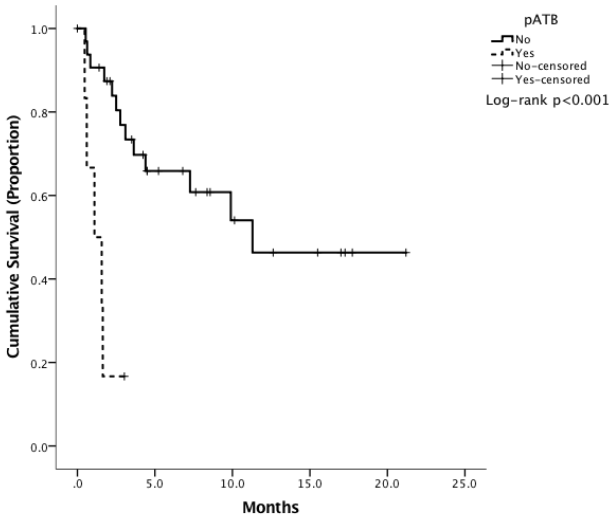
Characteristic	Propensity Score Weighted Model		Propensity Score Time-dependent Model	
	HR (95%CI)	**p-value**	**HR (95%CI)**	**p-value**
Response to ICPI CR/PR/SD/PD	9.0 (4.3-20.2)	**<0.001***	5.5 (3.0-10.0)	**<0.001***
pATB No/Yes	3.3 (1.9-5.9)	**<0.001***	2.6 (1.5-4.6)	**<0.001***
Associations reaching statistical significance (p<0.05) are marked with an asterisk (*).				

e**Figure 1.** The prognostic effect of prior antibiotic treatment (pATB) as a determinant of adverse survival in NSCLC (n=119, **Panel A**), Melanoma (n=38, **Panel B**) and other tumour types (n=38, **Panel C**).

A.



B.



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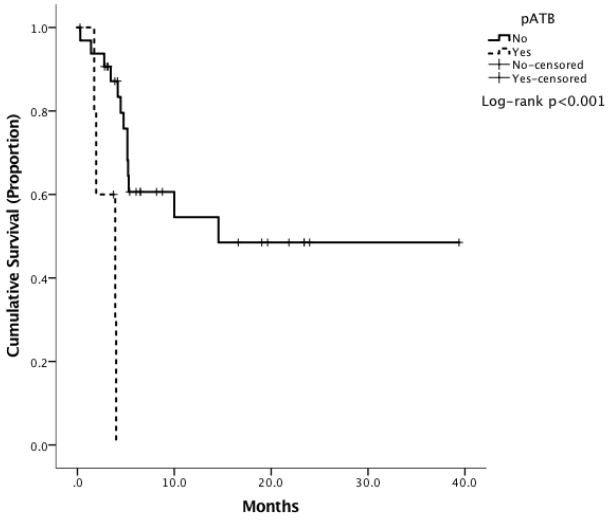
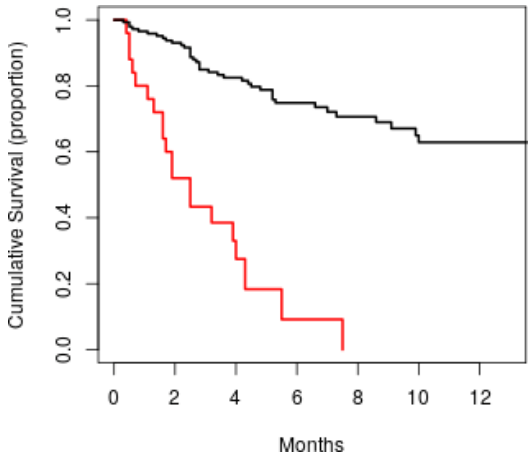


eFigure 2. Comparison between standard (**Panel A**) and inverse probability of treatment weighted Kaplan Meier plots (**Panel B**) illustrating the prognostic role of pATB in the whole study population receiving ICPI (n=196).

A.



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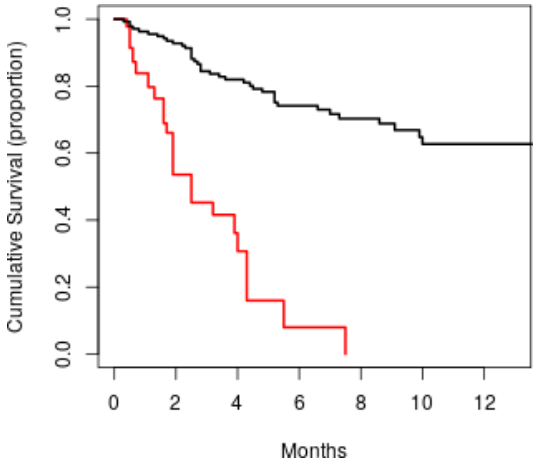
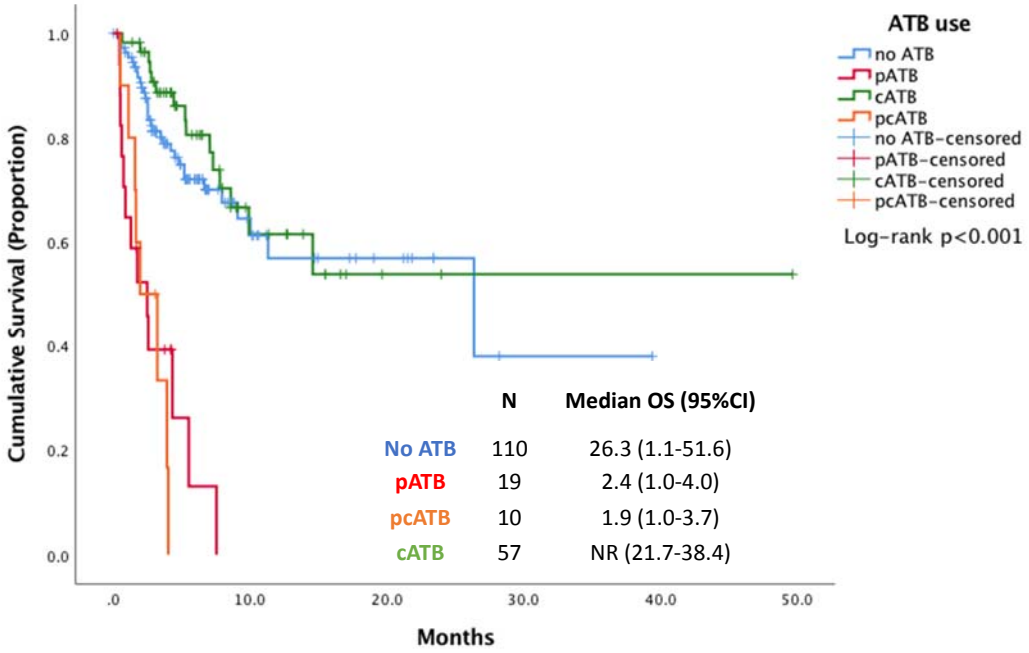
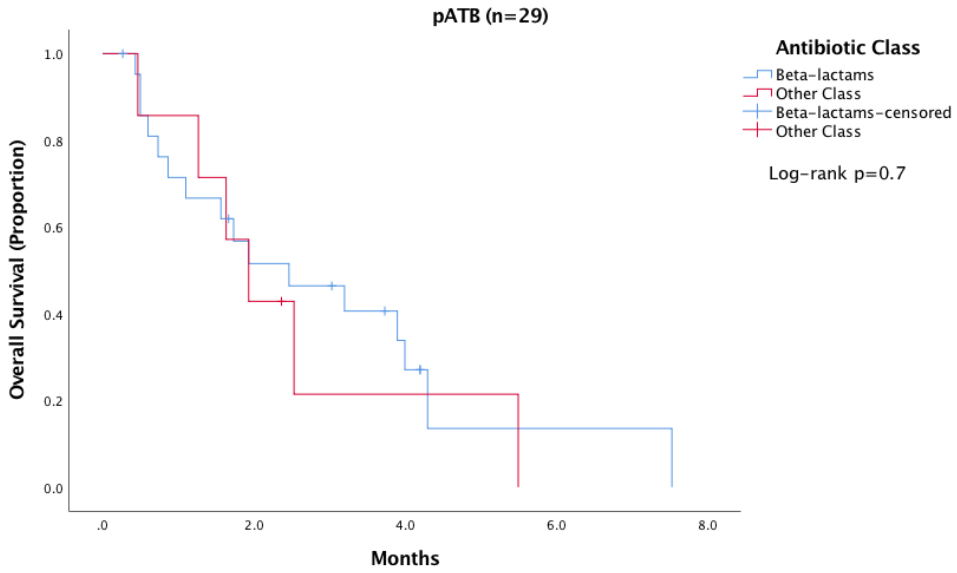


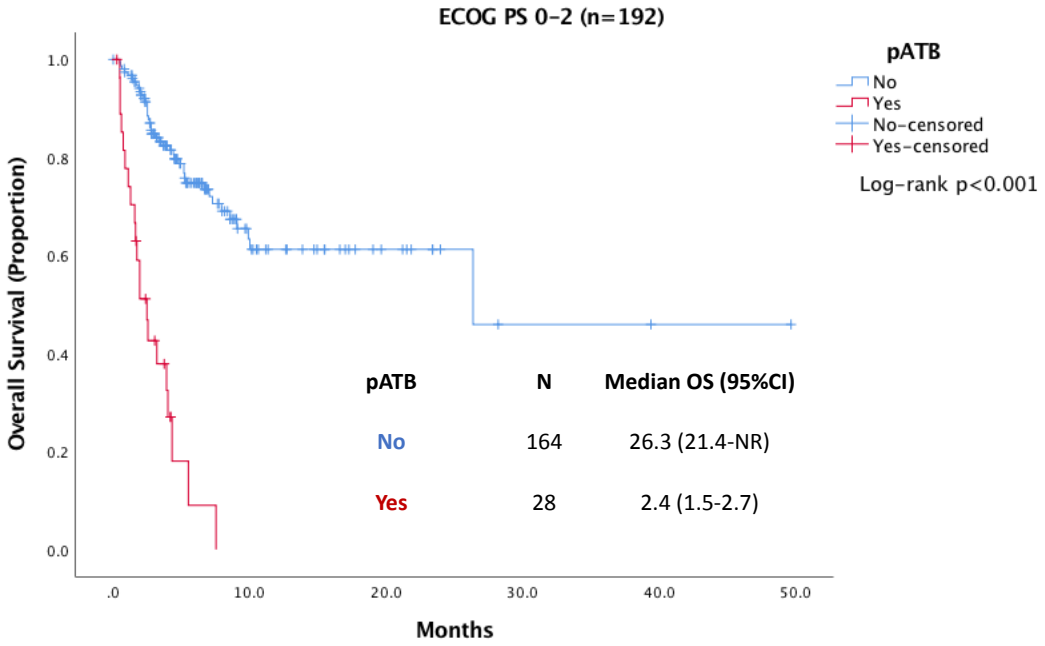
eFigure 3. The relationship between timing of antibiotic exposure and overall survival of patients treated with ICPI (n=196). Pairwise comparison across prognostic strata by Log-rank test revealed no significant difference in median OS between patients who did not receive antibiotics (No ATB, n=110) and cATB (n=57) groups (Chi-square 0.67, p=0.41) and between pATB (n=19) and patients who received ATB both prior to and during ICPI (pcATB, n=10, Chi-square 0.35, p=0.33). Strong evidence of differences were noted in the comparison of pATB and no ATB (Chi-square 31.6, p<0.001) and in pcATB and no ATB groups (Chi-square 25.3, p<0.001).



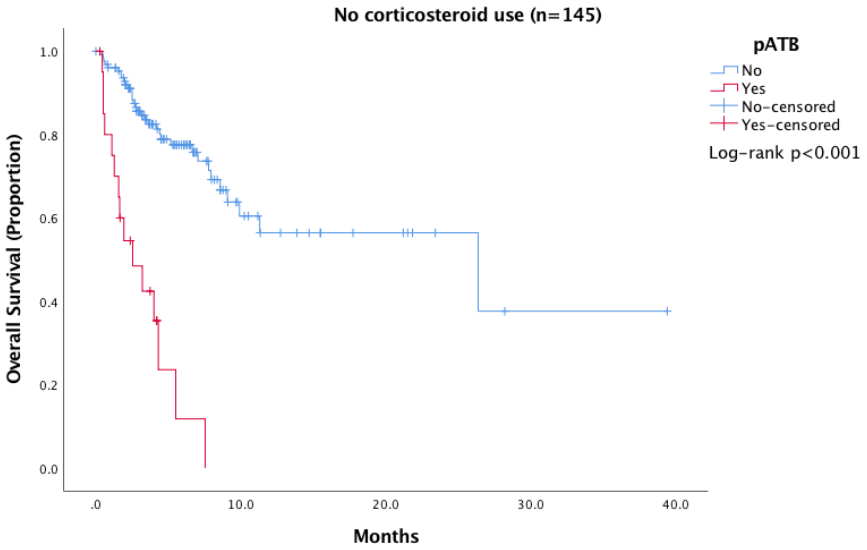
eFigure 4. The prognostic effect of antibiotic subtypes in patients receiving antibiotic therapy prior to ICPI (pATB, N=29).



eFigure 5. Verification of the prognostic role of pATB in patients with ECOG PS 0-2.



eFigure 6. pATB exerts detrimental effect on the prognosis of patients treated with ICPI irrespective of corticosteroids use.



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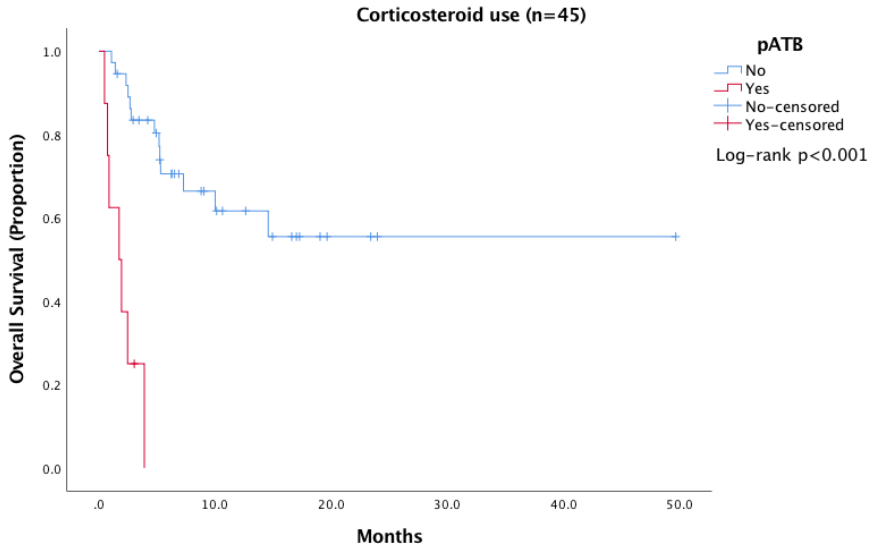

B.

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