Antibiotic Treatment is an Independent Poor Risk Factor in NSCLC But Not in Melanoma Patients Who had Received Anti-PD-1/L1 Monotherapy

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Abstract

Recent antibiotic treatment may reduce the efficacy of cancer immunotherapy. In retrospective study of 222 melanoma and 199 non-small-cell lung cancer patients who had been treated with anti-PD-1/L1 antibodies, antibiotic treatment was an independent risk factor for inferior overall survival and progression-free survival in NSCLC but not in melanoma when accounting for other relevant prognostic factors in multivariable analysis.

Background: Antibiotic treatment may reduce the efficacy of cancer immunotherapy by disrupting gut microbiome. We aimed to study the association of antibiotics and survival outcomes in advanced cutaneous melanoma and non–small-cell lung cancer (NSCLC) patients who had received anti-PD-1/L1 monotherapy. **Patients and Methods:** A total of 222 melanoma and 199 NSCLC patients had received anti-PD-1/L1 monotherapy in 5 Finnish hospitals between January 2014 and December 2020. Clinical characteristics, antibiotic and corticosteroid treatment, and survival outcomes were retrospectively collected from hospital and national medical records. **Results:** There were 32% of melanoma and 31% of NSCLC patients who had received antibiotic treatment (ABT) 3 months before to 1 month after the first anti-PD-1/L1 antibody infusion. In survival analyses, early antibiotic treatment was associated with inferior overall survival (OS) (ABT 19.2 [17.6-43.7] vs. no ABT 35.6 [29.3-NA] months, $P = .033$) but not with inferior progression-free survival (PFS) (ABT 5.8 [3.0-12.6] vs. no ABT 10.2 [7.7-15.3] months, $P = .3$) in melanoma patients and with inferior OS (ABT 8.6 [6.4-12.3] vs. no ABT 18.5 [15.1-21.6] months, $P < .001$) and PFS (ABT 2.8 [2.1-4.5] vs. no ABT 5.6 [4.4-8.0] months, $P = .0081$) in NSCLC patients. In multivariable analyses, ABT was not an independent risk-factor for inferior OS and PFS in melanoma but was associated with inferior OS (hazard ratio [HR] 2.12 [1.37-3.28]) and PFS (HR 1.65 [1.10-2.47]) in NSCLC after adjusted for other risk factors. **Conclusions:** Early ABT was an independent poor risk factor in NSCLC patients who had received anti-PD-1/L1 monotherapy but not in melanoma patients. The weight of ABT as a poor risk factor might depend on other prognostic factors in different cancers.

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Key Words: Antibiotics, Immune checkpoint inhibitors, Overall survival, Prognostic factor, Progression-free survival

Introduction

In Europe, anti-PD-1/L1 antibodies have been one of the mainstays of the treatment of advanced cutaneous melanoma and non–small-cell lung cancer (NSCLC) since 2015. In melanoma, the median progression-free survival (mPFS) was 5.1 to 8.4 months, and the median overall survival (mOS) 33 to 37 months with anti-PD-1 monotherapy outweighing the results of chemotherapy and ipilimumab.[1-3] In NSCLC, anti-PD-1/L1 antibodies were first shown

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to improve survival in patients previously treated with chemotherapy and later as the first-line treatment of tumors without EGFR mutations and ALK translocations.[4] The mPFS of 7.7 months, and the mOS of 26.3 months was observed in the first-line treatment of tumors with high PD-L1 expression (≥ 50%).[5] Anti-PD-1/L1 antibodies have also been successfully combined with chemotherapy to treat tumors with low PD-L1 expression (<50%).[6-9]

Gut microbiome has modulated the efficacy of anti-PD-1 antibodies in preclinical models.[10] Melanoma patients with high diversity fecal microbiome had longer PFS and certain commensal bacteria (*Bifidobacterium longum, Collinsella aerofaciens, and Enterococcus faecium*) were associated with better responses to anti-PD-1 therapy.[11,12] Thus, changes in gut microbiome after exposure to antibiotic treatment (ABT) might mediate the negative effect of ABT on the efficacy of cancer immunotherapy.

In retrospective studies, early ABT 3 months before to 1 month after the initiation of immune checkpoint inhibitors has been associated with inferior response rates, PFS, and OS in advanced melanoma, NSCLC, and renal cell carcinoma patients[13-18] whereas concurrent ABT during immunotherapy did not impair treatment outcomes.[14] ABT was also associated with shorter OS in completely resected stage III melanomas treated with adjuvant immunotherapy.[15] Patients with multiple courses of ABT had shorter PFS and OS compared to patients with single course or without ABT suggesting that the duration and the spectrum of ABT affect the efficacy of immunotherapy.[16]

Other prognostic factors might confound the effect of ABT on cancer immunotherapy. Shorter PFS and OS were observed in NSCLC patients with tumor PD-L1 expression ≥50% and early ABT but not in patients with PD-L1 expression <50% and early ABT.[19] Therefore, it is still controversial if ABT is an independent risk factor or just commonly used among patients with other poor prognostic factors.[13-18] In this study, we aimed to investigate if ABT is an independent risk factor for inferior outcomes of anti-PD-1/L1 monotherapy in advanced melanoma and NSCLC patients while accounting for other relevant risk factors.

Material and Methods

This study included patients from 4 university hospitals (Helsinki, Turku, Tampere, and Kuopio) and 1 central hospital (Vaasa) covering 85% of the population of Finland. The study investigators reviewed electronic medical records of all patients who had received anti-PD-1 monotherapy for advanced cutaneous or unknown primary melanomas (uveal and mucosal melanomas excluded) at Helsinki, Turku, Tampere, Kuopio, and Vaasa, as well as all advanced NSCLC patients who had received anti-PD-1/L1 monotherapy at Turku, Tampere, Kuopio, and Vaasa between January 1, 2014, and December 31, 2020.

Patient characteristics at the time of the first anti-PD-1/L1 antibody infusion were collected along with the information on radiological response, disease progression, and OS. The use of ABT (indication, class, and duration) 3 months before the initiation and during anti-PD-1/L1 therapy and corticosteroid treatment (oral prednisolone >10 mg daily, oral dexamethasone, and intravenous methylprednisolone) during anti-PD-1/L1 therapy were manually obtained from the electronic medical records of each study hospital and from national electronic medical records to cover visits outside hospitals.

Study Objectives

The primary objective of our study was to analyze the association of ABT with PFS and OS of melanoma and NSCLC patients treated with anti-PD-1/L1 monotherapy. The effect of early ABT (the use of antibiotics within 1 to 3 months before and after the initiation of immunotherapy) has been most widely studied. Therefore, we decided to determine ABT patients as the group of patients who had received antibiotics 3 months before to 1 month after the first anti-PD-1/L1 antibody infusion.

Statistical Analysis

The results of continuous variables are presented as median (range) and those of categorical variables as numbers and percentages. OS was measured from the first anti-PD-1/L1 antibody infusion to the date of death or the last follow-up visit. PFS was calculated similarly to the date of disease progression, death, or the last follow-up visit. Kaplan-Meier curves were used to illustrate survival analyses of PFS and OS. Kaplan-Meier estimates of OS and PFS are presented with 95% confidence intervals (95% CI) and the log-rank test was used to calculate statistical significance.

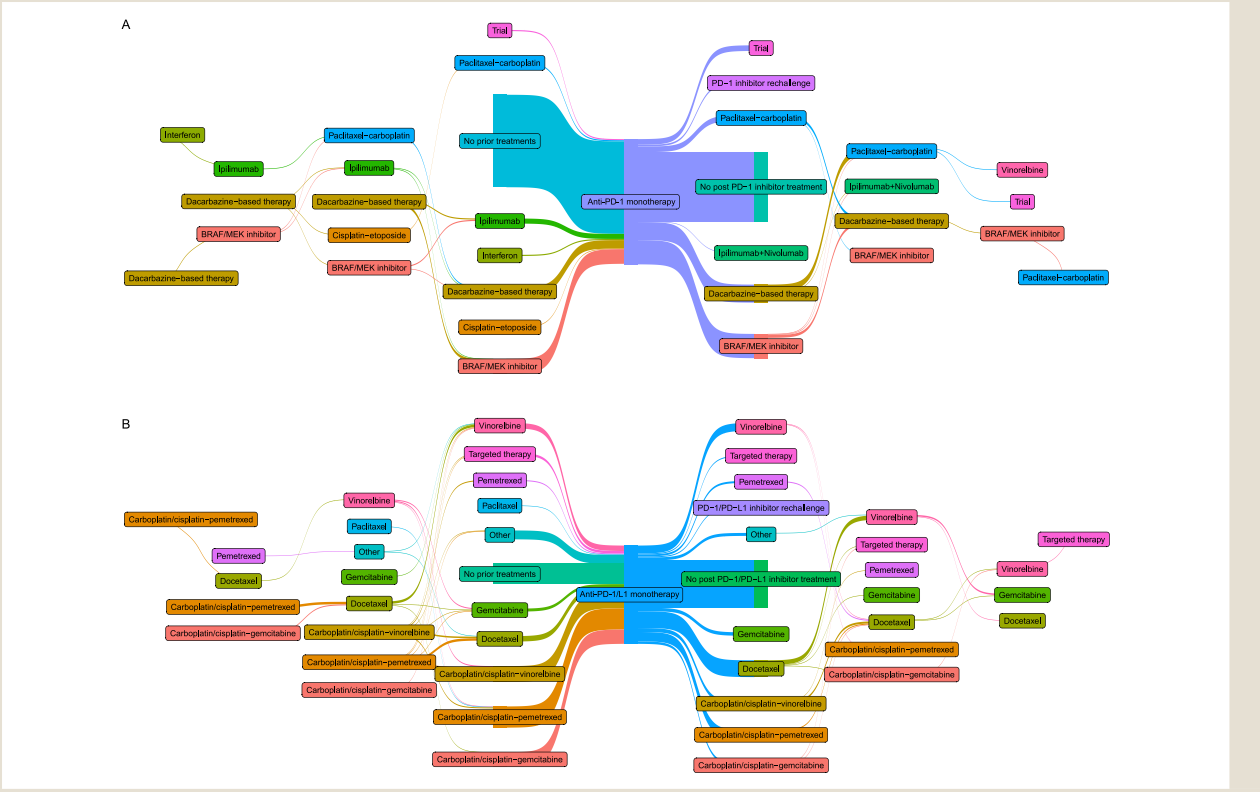
The multivariable Cox regression analysis was performed to study if early ABT is an independent risk factor for inferior PFS and OS after adjusted for other relevant risk factors. Analyses were performed separately for melanoma and NSCLC patients because of differences in relevant prognostic factors and outcomes of cancer immunotherapy. In melanoma, other prognostic factors included sex (male, female), ECOG performance status (0-1, ≥2), age (≥65 years, <65 years), Charlson comorbidity index score, treatment line (first-line, later line), corticosteroid treatment (yes, no), BRAF mutation status (BRAF V600 mutated, no known mutation), lactate dehydrogenase (LDH) level (elevated, normal), and stage according to AJCC 8th edition (M1a, M1b, M1c, M1d). In NSCLC, sex, ECOG performance status, age, Charlson comorbidity index score, smoking status (ever-smoker, never-smoker), treatment line, corticosteroid treatment, histological type (adenocarcinoma, other, squamous cell carcinoma), C-reactive protein (CRP) level (normal, elevated), PD-L1 expression of tumor cells (≥50%, <50%), brain metastases (yes, no), and stage according to AJCC 8th edition (stage III, M1a, M1b, M1c) were included into the multivariable Cox model along with early ABT. While there were missing values in the risk factors, their proportion was assessed to be so small that imputation was not required and effective N in the final multivariable models were the fully observed portions of data.

All statistical analyses and visualizations were performed with R statistical software (version 4.2.0; R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Ethical Approval Statement

This study was approved by the institutional review boards of Helsinki (license HUS/239/2017), Turku (license T88/2020), Tampere (license R20618F), Kuopio (license 5654213 [TJ 8/2020,

Figure 1 Prior and subsequent cancer treatments in melanoma (panel A) and NSLCL patients (panel B) who had received anti-PD-1/L1 monotherapy.



RPL 51/2020]), and Vaasa (license VKS–JYL/25/2020) hospitals. Data was anonymized before statistical analyses and handled in a manner that met general regulations on data protection.

Results

Patient Characteristics

There were 222 advanced cutaneous melanoma patients and 199 advanced NSCLC patients who had been treated with anti-PD-1/L1 monotherapy during 2014-2020 (Table 1). 164 (74%) melanoma patients had received anti-PD-1 antibodies for first-line treatment and 58 (26%) during later treatment lines (Figure 1A). 45 (23%) NSCLC patients had received anti-PD-1/L1 antibodies as first-line treatment and 154 (77%) during later treatment lines (Figure 1B). 38 (84%) first-line patients had PD-L1 ≥50% compared to 76 (49%) later line patients. 71 (32%) melanoma and 61 (31%) NSCLC patients had received early ABT in our study. There was not a statistically significant difference in the exposure to early ABT in different treatment lines in melanoma (early ABT 48 [29%] first-line treatment vs. early ABT 23 [40%] later line treatment, χ^2 test $P = .1957$) or in NSLCL patients (early ABT 12 [27%] first-line treatment vs. early ABT 49 [32%] later line treatment, $P = .6344$). In addition, 67 (30%) melanoma and 56 (28%) NSCLC patients had been treated with corticosteroids during anti−PD-1/L1 monotherapy, typically because of immune related adverse events.

Continuous values are reported as median (min–max) and categorical/ordinal variables as counts (percentages).

Treatment Outcomes

The median follow-up times (until censoring or death) were 21.9 (first-line treatment) and 17.6 months (later line treatment) in melanoma patients and 17.3 and 14.6 months in NSCLC patients, respectively. Survival outcomes of anti-PD-1/L1 monotherapy were better in advanced melanoma patients compared to NSCLC patients. First-line patients had longer median survival times in comparison with later line patients in both cancers. In melanoma, the mPFS was 10.83 (7.1-15.6) months and the mOS was 31.6 (25.0-45.0) months with first-line anti-PD-1 monotherapy. The mPFS was 4.77 (2.1-10.3) months and the mOS 23.5 (9.63-NA) months with later line anti-PD-1 monotherapy. In NSCLC, the mPFS was 7.4 (4.87-12.7) months and the mOS was 21.1 (14.8-NA) months with first-line anti-PD-1/L1 monotherapy. The median PFS was 3.9 (2.57-5.5) months and the median OS 14.6 (11.5-17.7) months with later line anti-PD-1/L1 monotherapy.

Early ABT and Treatment Outcomes

In melanoma patients, early ABT was associated with worse OS (mOS early ABT 19.2 [17.6-43.7] vs. no ABT 35.6 [29.3-NA] months, hazard ratio [HR] 1.53 [1.03-2.26], log-rank test $P = .033$,

Table 1 Clinical Characteristics and Survival Outcomes of Advanced Melanoma and NSCLC Patients

Variable	Melanoma n=222	Variable	NSCLC n=199
Median Age years (–min–max)	65 (24–83)	Median Age years (–min–max)	68 (37–81)
Male	131 (59%)	Male	133 (67%)
Female	91 (41%)	Female	66 (33%)
Median Charlson comorbidity index (–min–max)	6 (4–13)	Median Charlson comorbidity index (–min–max)	9 (6–13)
ECOG 0	128 (58%)	ECOG 0	40 (20%)
ECOG 1	86 (39%)	ECOG 1	126 (63%)
ECOG ≥2	8 (4%)	ECOG ≥2	33 (17%)
LDH normal	122 (55%)	CRP normal	80 (40%)
LDH elevated	88 (40%)	CRP elevated	106 (53%)
LDH unknown	12 (5%)	CRP unknown	13 (7%)
BRAF V600 positive	90 (41%)	Targetable mutation	11 (6%)
BRAF V600 negative	127 (57%)	No mutation or unknown	188 (94%)
BRAF status unknown	5 (2%)		
M1a	59 (27%)	stage III	37 (19%)
M1b	55 (25%)	M1a	66 (33%)
M1c	85 (38%)	M1b	30 (15%)
M1d (brain metastases)	23 (10%)	M1c	66 (33%)
		With brain metastases	13 (7%)
		Without brain metastases	186 (93%)
		Current smoker	83 (42%)
		Ex-smoker	101 (51%)
		Never-smoker	13 (7%)
		Smoking status unknown	2 (1%)
		PD-L1 high (≥50%)	114 (57%)
		PD-L1 low (<50%)	51 (26%)
		PD-L1 status unknown	34 (17%)
		Adenocarcinoma	106 (53%)
		Squamous cell carcinoma	83 (42%)
		Other histology	9 (5%)
		Unknown histology	1 (1%)
First-line anti-PD-1	164 (74%)	First-line anti-PD-1/L1	45 (23%)
Later line anti-PD-1	58 (26%)	Later line anti-PD-1/L1	154 (77%)
Corticosteroid treatment	67 (30%)	Corticosteroid treatment	56 (28%)
No corticosteroid treatment	155 (70%)	No corticosteroid treatment	142 (71%)
Corticosteroid treatment unknown	0 (0%)	Corticosteroid treatment unknown	1 (1%)
Early ABT	71 (32%)	Early ABT	61 (31%)
No ABT	151 (68%)	No ABT	138 (69%)
Anti-PD-1 therapy		Anti-PD-1/L1 therapy	
Nivolumab	120 (54%)	Nivolumab	61 (31%)
Pembrolizumab	100 (45%)	Pembrolizumab	89 (45%)
Nivolumab and pembrolizumab[a]	2 (1%)	Atezolizumab	46 (23%)
		Durvalumab	3 (2%)
Survival outcomes		Survival outcomes	
OS, death observed	108 (49%)	OS, death observed	138 (69%)
OS, alive	111 (50%)	OS, alive	61 (31%)
OS status unknown	3 (1%)	OS status unknown	0 (0%)
PFS, PD or death observed	156 (70%)	PFS, PD or death observed	163 (82%)
PFS, alive without PD	61 (27%)	PFS, alive without PD	36 (18%)
PFS status unknown	5 (2%)	PFS status unknown	0 (0%)

PD = disease progression.
[a] nivolumab switched to pembrolizumab or vice versa,

Figure 2 OS and PFS of anti-PD-1/L1 monotherapy by early ABT in melanoma (panels A-B) and NSCLC (panels C-D).

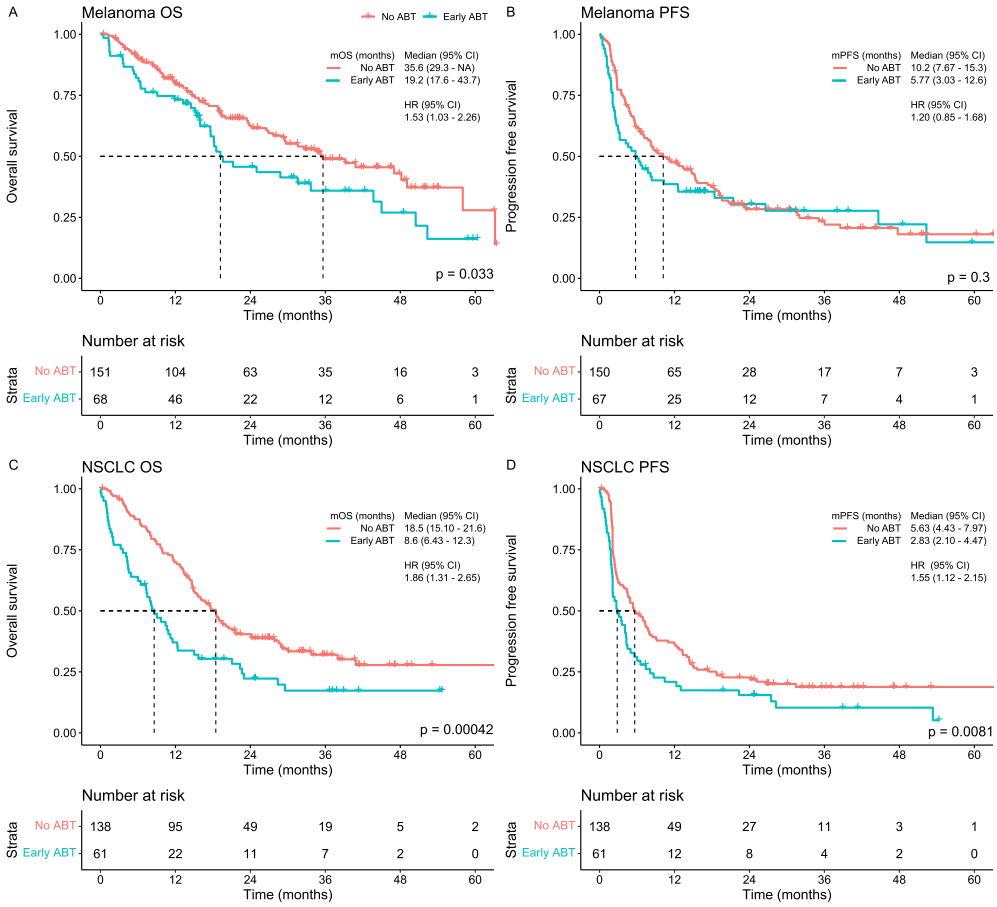


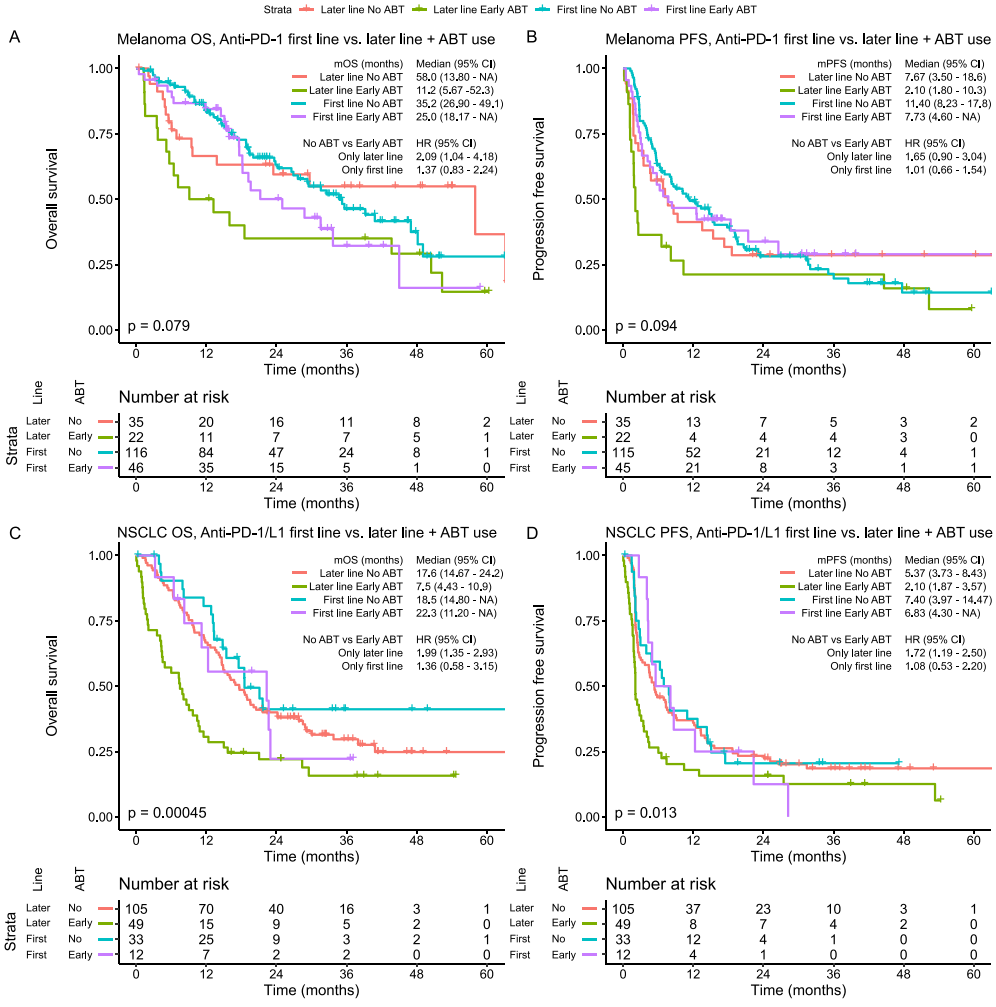
Figure 2A) but was not associated with worse PFS (mPFS early ABT 5.8 [3.0-12.6] vs. no ABT 10.2 [7.7-15.3] months, HR 1.20 [0.85-1.68], $P = .3$, Figure 2B). In NSCLC patients, early ABT was associated with worse OS (mOS early ABT 8.6 [6.4-12.3] vs. no ABT 18.5 [15.1-21.6] months, HR 1.86 [1.31-2.65], $P < .001$, Figure 2C) as well as with worse PFS (mPFS early ABT 2.8 [2.1-4.5] vs. no ABT 5.6 [4.4-8.0] months, HR 1.55 [1.12-2.15], $P = .0081$, Figure 2D).

Early ABT had a negative impact on OS and PFS, especially in later-line anti-PD-1/L1 monotherapy with near borderline statistical significance in melanoma patients (OS $P = .079$, Figure 3A and PFS $P = .094$, Figure 3B) and highly statistically significant association in NSCLC patients (OS $P < 0.001$, Figure 3C and PFS $P = 0.013$, Figure 3D). There were no differences in OS and PFS according to ABT-use in the subgroup of patients with first-line anti-PD-1/L1 therapy in melanoma and NSCLC. However, there was a statistically significant difference in the OS of patients with early ABT vs no ABT in the subgroup of melanoma patients with later line anti-PD-1 therapy (HR of death 2.09 [1.04-4.18]), Figure 3A). There were statistically significant differences in the OS (HR of death 1.99 [1.35-2.93]) and the PFS (HR of disease progression or death 1.72 [1.19-2.50]) of patients with early ABT vs. no ABT in the subgroup of NSCLC patients with later line anti-PD-1/L1 therapy (Figure 3C and D).

In multivariable Cox regression analysis, early ABT was not an independent risk factor for inferior PFS and OS in melanoma patients after addressing potential confounding factors (Figure 4). Elevated LDH and brain metastases (M1d) were associated with inferior PFS in melanoma. Male sex, corticosteroid treatment during immunotherapy, visceral metastases outside lungs and central nervous system (M1c), and brain metastases (M1d) were associated with inferior OS in melanoma. Impaired performance status (ECOG PS $\geq$2) was also associated with inferior OS in melanoma with borderline statistical significance.

Early ABT was an independent risk factor for inferior PFS (HR of PD or death 1.65 (1.10-2.47) and OS (HR of death 2.12 [1.37-3.28] in NSCLC patients in multivariable analysis (Figure 5). Male sex, age $\geq$65 years, ever-smokers, and corticosteroid treatment during immunotherapy were associated with improved PFS, whereas early ABT brain metastases, stage M1a, and stage M1c were associated with inferior PFS in NSCLC. First-line treatment with anti-PD-1/L1 monotherapy was associated with improved OS, whereas

Figure 3 OS and PFS in first-line and later-line treatment by early ABT in melanoma (panels A-B) and NSCLC (panels C-D).



early ABT, elevated CRP, brain metastases, stage M1a, stage M1b, and stage M1c were associated with inferior OS.

Discussion

Anti-PD-1/L1 antibodies belong to the standard treatment of advanced melanoma and NSCLC. However, some tumors are primarily refractory, and some develop acquired resistance against immunotherapy. Gut microbiome interacts with immune system and may also play a role in cancer immunotherapy. Fecal transplants from immunotherapy responders have even induced responses among initially immunotherapy refractory melanoma patients.[20,21]

In previous studies, the use of antibiotics has been associated with inferior benefit from immunotherapy in different advanced cancers[13-18] and the negative effect is suggested to be mediated by the transformation of gut microbiome.[10-12] ABT is used for cancer patients with symptoms related to bacterial infection, such as fever and elevated CRP, often without verification of bacterial disease. In an Australian study, 56% of the episodes of suspected infections in cancer patients were microbiologically or clinically documented but the remaining 44% had no focus on infection.[22]

In this study, early ABT was associated with inferior OS in melanoma and with inferior PFS and OS in NSCLC patients who had received anti-PD-1/L1 monotherapy. The negative effect was most pronounced in later treatment lines. Similar proportion (31%-32%) of melanoma and NSCLC patients had received ABT within 3 months before to 1 month after the first anti-PD-1/L1 antibody infusion which is also comparable to the rates of 18.5 to 39% observed in previous studies.[13-18] We did not find a statistically significant difference in the proportion of patients who had received early ABT in first-line or later-line treatment.

In multivariable Cox model, early ABT was an independent poor risk factor doubling the risk of disease progression and death in NSCLC patients when accounting for relevant prognostic factors. However, early ABT was not significantly associated with inferior OS and PFS in melanoma patients in multivariable analysis. This suggests that the weight of ABT as a poor risk factor might be

Figure 4 Multivariable Cox model for OS (panel A) and PFS (panel B) in melanoma patients.

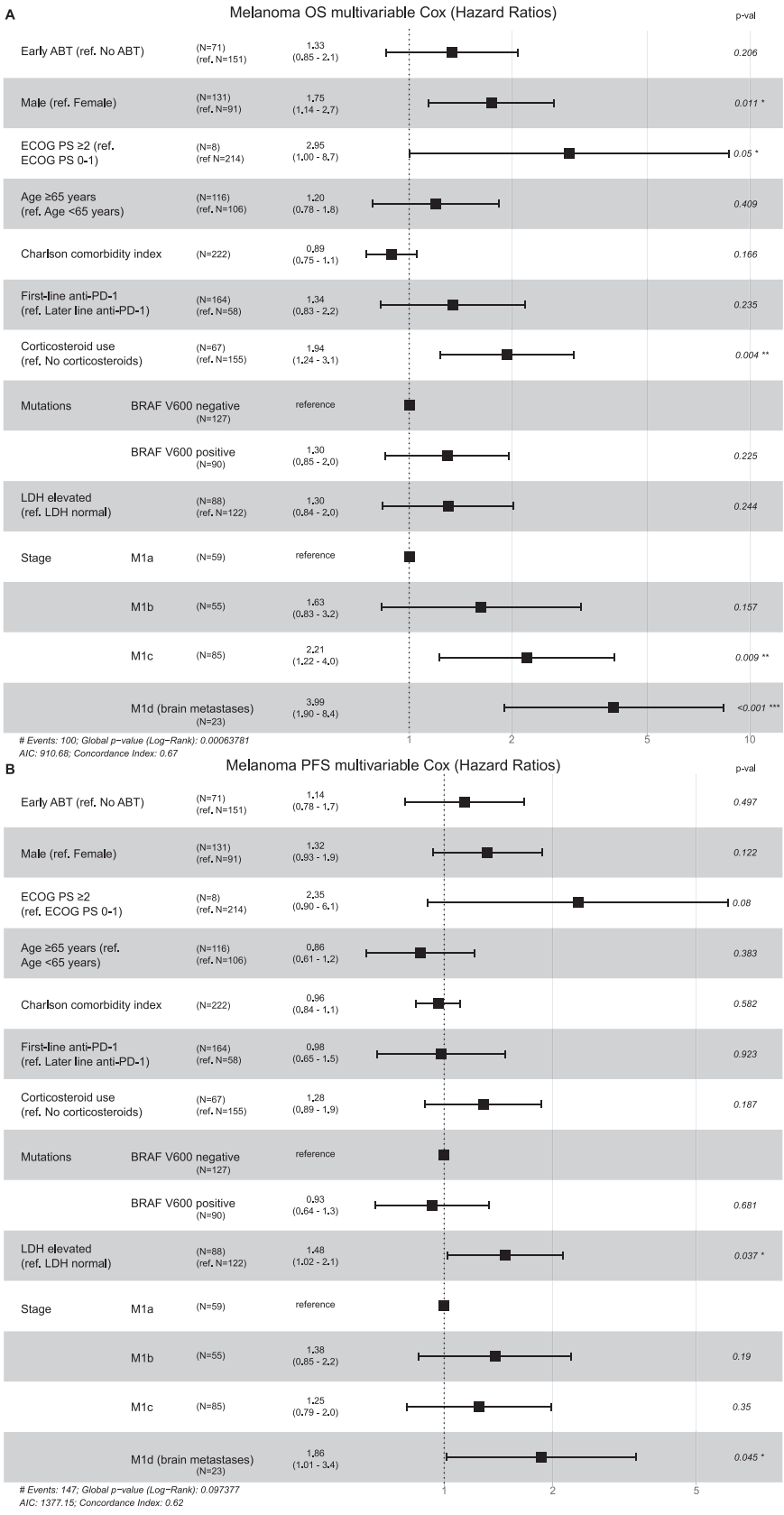
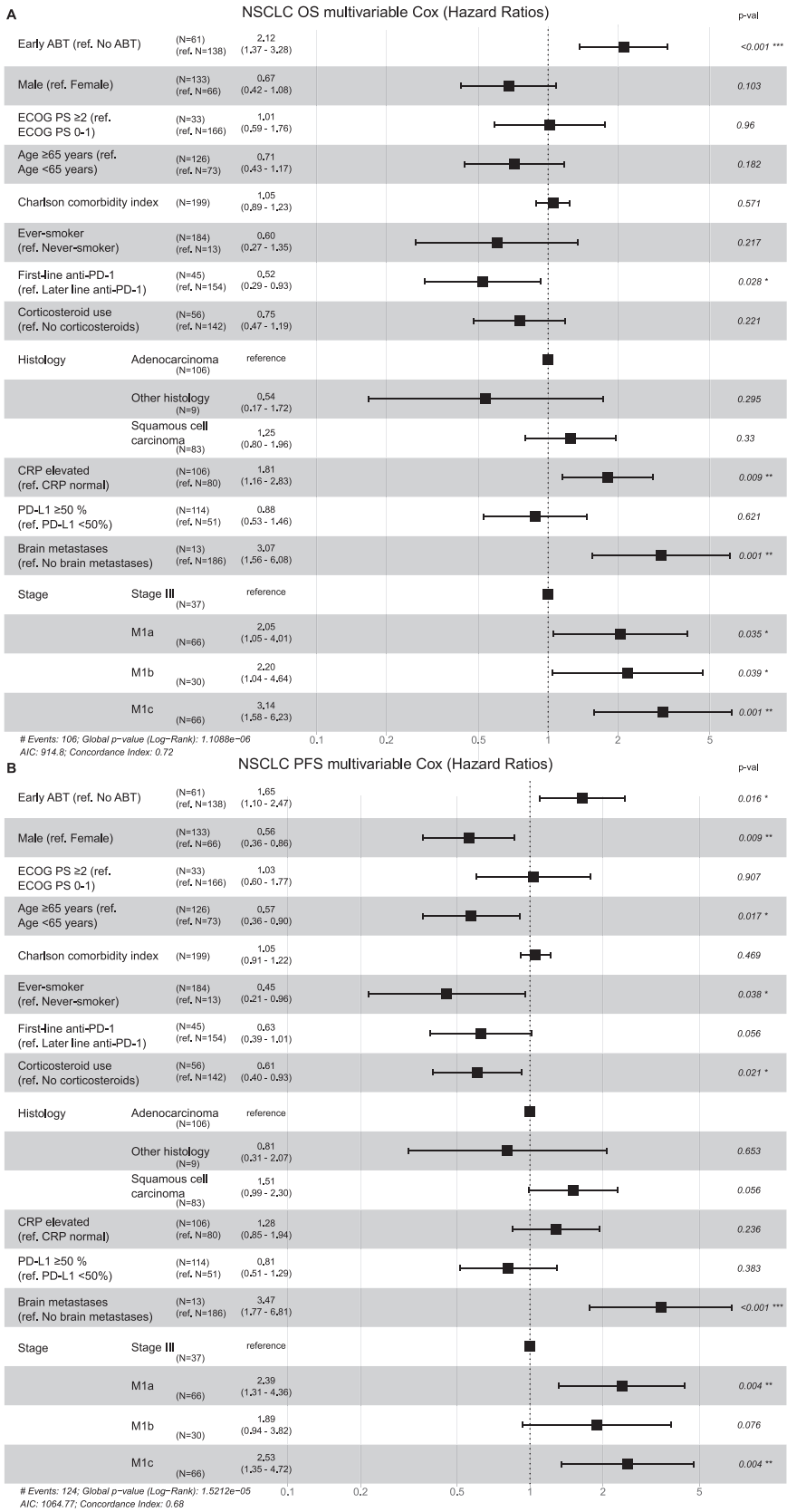


Figure 5 Multivariable Cox model for OS (panel A) and PFS (panel B) in NSCLC patients.



confounded by other prognostic factors and the effect of ABT on immunotherapy might be different across different cancers and treatment lines. In the first-line treatment of NSCLC patients, the effect of ABT has depended on PD-L1 expression levels.[19] In this study, PD-L1 expression levels were not associated with PFS and OS in multivariable analysis which could have been explained by the larger proportion of later line patients. In addition to early ABT, brain metastases and more advanced stages were poor risk factors in NSCLC. In melanoma patients, other prognostic factors such as male sex, elevated LDH, more advanced stage, and corticosteroid treatment seemed to be more relevant to poor risk factors than early ABT according to our findings.

The limitations of this study are attributed to the retrospective collection of study data. Study patients were treated within routine clinical practice. Therefore, response evaluation with thoracic and abdominal CT was performed according to local follow-up guidelines which could have affected PFS results. The use of anti-PD-1/L1 antibodies was comprehensively obtained from hospital medical records as well as the use of corticosteroid and antibiotic treatment in each study hospital. Antibiotics prescribed outside hospital visits were manually searched from national electronic medical records. Therefore, we might have missed some prescriptions. The use of anti-PD-1/L1 antibodies reflects the clinical practice during the study period from 2014 to 2020 and differ greatly between melanoma and NSCLC. There has been a shift towards earlier use of immunotherapy as well as towards the use of combination therapies instead of anti-PD-1/L1 monotherapy. At this stage, we did not evaluate the duration of ABT and the use of broad-spectrum antibiotics as risk factors, and this will be the target of our further analyses. Because of the lack of fecal and blood samples during immunotherapy from our patients, the direct effect of ABT on patients‘ gut microbiome and immune cells was not possible to analyze. Prospective evaluation of fecal and blood samples during cancer immunotherapy will shed light on this question.

Conclusions

There are lots of intertwined risk factors affecting outcomes of cancer immunotherapy. According to this study, early ABT was an independent risk factor for inferior PFS and OS in NSCLC patients who had received anti-PD-1/L1 monotherapy but not in melanoma patients. The use of antibiotics should be weighed against potential negative impact on cancer immunotherapy and patients who have recently received ABT may need more than anti-PD-1/L1 monotherapy for cancer treatment.

Clinical Practice Points

- Recent antibiotic treatment (ABT) has been shown reduce the efficacy of cancer immunotherapy in different cancer types including melanoma, non–small-cell lung cancer (NSCLC), and kidney cancer. The negative effect of ABT on treatment results is suggested to be mediated by detrimental changes in gut microbiome. There is still a controversy if ABT is an independent poor risk factor or commonly used among patients with other poor risk factors.
- In this study, the association of ABT and survival outcomes were retrospectively analyzed in melanoma and NSCLC patients who had received anti-PD-1/L1 antibodies. Early ABT 3 months before to 1 months after the first anti-PD-1/L1 antibody infusion was an independent poor risk factor doubling the risk of disease progression and death in NSCLC patients irrespective of other prognostic factors including performance and smoking status, histological type, PD-L1 expression, and CRP levels. However, early ABT was not associated with inferior OS and PFS in melanoma patients while accounting for age, sex, performance status, LDH levels, and BRAF mutation status. This suggests that the weight of ABT as a poor risk factor might be confounded by other risk factors in different cancer types.
- The current study adds to evidence that ABT is a poor prognostic factor in NSCLC patients who are eligible for immunotherapy. The use of antibiotics should be weighed against potential negative impact on cancer immunotherapy and patients who have recently received ABT may need more than anti-PD-1/L1 monotherapy for cancer treatment.

Disclosure

TK has received consulting or advisory honoraria from Eli Lilly, Bristol-Myers Squibb, Merck, Novartis and Daiichi Sankyo; LT has received consulting or advisory honoraria from Amgen, Bristol-Myers Squibb, Merck, Novartis, Pierre-Fabre, Pfizer, Roche, and Sanofi; TS has received consulting or advisory honoraria from Astra Zeneca, Merck, Bristol-Myers Squibb, and Faron; ST has received advisory board honoraria from Novartis and speaker honoraria from Finnish Melanoma group, society of Finnish outpatient clinic nurses, society of Finnish cancer nurses, and Bristol-Myers Squibb; MH has received consulting or advisory honoraria from Merck, Bristol-Myers Squibb, Incite, Varian, Novartis, and Roche; speakers' bureau honoraria from Merck, Novartis, and Bristol-Myers Squibb; MS has received consulting or advisory honoraria from MSD, Bristol-Myers Squibb, Takeda, Roche, AstraZeneca, Amgen, Novartis, Merck, and Janssen; KEM has received consulting or advisory honoraria from Astellas, Bayer, Bristol-Myers Squibb, GlaxoSmithKline, Ipsen, Janssen, Merck Sharp & Dohme, Merck–Pfizer alliance, Novartis, Roche, and Sanofi; HV, AJ, TDL, NW, LP, TK, AR, HK have no conflicts of interest to declare.

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Data availability

Data is available upon reasonable request from the corresponding author.

Author contributions

HV, AJ, and KEM had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: KEM. Acquisition of data: HV, AJ, KEM, NW, LP, TK, AR, LT, TS, LK, SK, HK, MH. Analysis and interpretation of data: TDL, HV, AJ, KEM. Drafting of the manuscript HV, AJ, TDL, NW, LP, TK, AR, LT, TS, LK,

SK, HK, MH, MS, KEM. Statistical analysis: TDL, HV, AJ, KEM. Obtaining funding: KEM. Supervision: KEM, LT, TS, MH.

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