The Association Between Antibiotic Use and Outcome Among Metastatic Melanoma Patients Receiving Immunotherapy

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Abstract

Background: Several observational studies have reported a decreased response to immune checkpoint inhibitors (ICI) following antibiotic use. ICI activity has been hypothesized to be impaired by antibiotic-induced gut dysbiosis.

Methods: Patients with advanced melanoma receiving an anti-PD-1 antibody as a first-line therapy between 2015 and 2017 in France were selected using the French Health Insurance database. We compared overall survival (OS) and time-to-treatment discontinuation (TTD) according to antibiotic exposure in the 3 months prior to the initiation of anti-PD-1 antibody. To disentangle a causal effect of antibiotics from a confounding bias, we balanced characteristics of patients exposed and non-exposed to antibiotics using an overlap weighting method based on a propensity score. We also evaluated a control cohort of patients with advanced melanoma receiving first-line targeted therapy, as there is no rationale for decreased efficacy of targeted therapy following antibiotic treatment.

Results: The anti-PD-1 antibody cohort comprised 2605 individuals. Antibiotic exposure in the 3 months prior to anti-PD-1 antibody initiation was not associated with shorter OS (weighted hazard ratio = 1.01, 95% confidence interval = 0.88-1.17) or TTD (weighted hazard ratio = 1.00, 95% confidence interval = 0.89-1.11). Consistent results were observed when the timeframe of antibiotics was narrowed to 1 month prior to anti-PD-1 initiation, or when exposure was restricted to antibiotics leading to more profound gut dysbiosis. Similar results were observed in the targeted therapy cohort.

Conclusions: In a large cohort of advanced melanoma patients, we showed that antibiotic use preceding anti-PD-1 antibody was not associated with worse outcome. Physicians should not delay immunotherapy for patients who have recently received antibiotics.

Tumor immunotherapy using immune checkpoint inhibitors (ICI) has dramatically changed the prognosis of metastatic melanoma as well as several other cancers.[1] However, only approximately 35% to 45% of patients benefit from immunotherapy.[2–4] Identification of factors that affect the efficacy of ICI has therefore become a new challenge. One of the most disturbing findings that has emerged in the past 3 years is the suspected detrimental effect of antibiotics: across several cancers, antibiotics administered before ICI have been associated with shorter progression-free and overall survival.[5–11] Because the role of the gut microbiota in response to ICI has been substantiated by experimental data in mice,[12] the hypothesis of an impairment of ICI efficacy consecutive to antibiotic-induced gut dysbiosis has been raised.[13] Among patients receiving ICI, dysbiosis could consecutively lead to cancer progression and death. Alongside this neat causal hypothesis, however, one alternative line of explanation, namely confounding by indication, has not been ruled out, nor even appropriately discussed in the vast majority of reports. Thus, more aggressive disease or a comorbid burden among patients receiving antibiotics could lead to the poorer prognosis observed, independently from any causal effect of antibiotic exposure.[14,15] In other words, patients with more active or advanced metastatic cancer, with a poorer prognosis, are more likely to require medical care, including antibiotic courses. Because it is very challenging to control for, confounding by indication has been considered as the "most stubborn bias".[16]

We addressed the question of the impact of exposure to antibiotics on ICI efficacy and survival in a cohort of advanced melanoma patients using data prospectively collected in the French Health Insurance database. To ensure the best comparability between patients exposed and non-exposed to antibiotics, we used a propensity score weighting method, and included a large homogeneous single-cancer population. We defined two cohorts according to the first-line treatment used: anti-PD-1 antibody or targeted therapy. The hypothesis of a deleterious effect of

antibiotic-induced gut dysbiosis has been raised for ICI, although no suspicion of such an effect literature has emerged in the literature on targeted therapy. Therefore, studying these two cohorts in parallel offered the opportunity to disentangle causal effect from bias.

Methods

Data source and study cohorts

This study was conducted using the French National Health Insurance database (SNDS, Système National des Données de Santé),[17,18] and followed the REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) guidelines.[19] The database covers 98.8% of the population living in France (around 66 million inhabitants), and contains exhaustive data on all reimbursements for health-related expenditures, including dispensed drugs with date of dispensation. Information about all hospitalizations in a public or private hospital is also provided, including diagnoses (using ICD-10 codes, International Classification of Diseases, 10th revision) and expensive drugs prescribed during hospital stays. Long-term diseases (including cancers) are recorded, with diagnoses encoded according to ICD-10, because they give entitlement to 100% health insurance coverage.

We previously constructed a national cohort including every new patient receiving a systemic treatment for metastatic melanoma in France between June 2015 and December 2017 (N=4725).[20] The population selection process has been detailed previously.[20] We selected two cohorts of patients with metastatic melanoma, differentiated by the first-line treatment received: an anti-PD-1 antibody cohort including 2605 patients receiving pembrolizumab or nivolumab,

and a targeted therapy cohort including1527 patients receiving BRAF inhibitors (vemurafenib, dabrafenib), alone or combined with MEK inhibitors (cobimetinib, trametinib).

Ethics approval

The study was approved by the French Data Protection Agency (CNIL, DR-2016-384).

Drug exposure

A timeframe for antibiotic courses received in the 3 months prior to the initiation of the first-line anti-melanoma treatment was chosen for the main analysis, so as to correspond to the previous literature regarding antibiotic exposure and ICI efficacy among patients with melanoma.[7] Antibiotic exposure was ascertained from dispensations occurring outside the hospital and was assumed to last 7 days, except for certain packages of tetracyclines used for acne and rosacea (60 days), single-dose fosfomycin (1 day), and benzathine penicillin G (14 days).

Sensitivity analyses

In a first sensitivity analysis, we narrowed the timeframe for antibiotic exposure to 1 month prior to the first-line anti-melanoma treatment, as this timing appeared as more detrimental for ICI efficacy.[9,11] In a second sensitivity analysis, antibiotic exposure was restricted to antibiotics having a higher impact on the gut microbiota, namely fluoroquinolones and broad-spectrum β-lactams (including second- and third-generation cephalosporins, and penicillins associated with β-lactamase inhibitors).[21–24] These can be considered as the backbone for testing the hypothesis of impaired efficacy of ICI as a result of antibiotic-induced gut dysbiosis. Patients receiving high-impact antibiotics were compared to patients who did not receive antibiotics. A third sensitivity analysis was performed because administration of antibiotics is not recorded during hospital stays

in the SNDS database. Patients with an infection identified in hospital discharge coding, who could have been misclassified in the "no antibiotics" group if they had received antibiotics only during their hospital stay, were excluded.

Outcomes

Two outcomes were used. Overall survival (OS) was estimated from the initiation of the first treatment line to the date of death or until censoring on December 31, 2017. The duration of the first treatment line, estimated from time-to-treatment discontinuation (TTD), was defined as the date of the start of the first treatment line to the date of treatment discontinuation. The date of the start of the first treatment line was the date of the first dispensation of targeted therapy or infusion of anti-PD-1 antibody. The treatment line was considered as discontinued when another treatment line was initiated, or 3 months after the last recorded dispensation if no subsequent treatment was initiated. TTD has been proposed as an efficacy end-point for real-world evidence trials.[25,26]

Covariates

Multiple covariates were used: age, sex, number and location of metastatic sites, previous surgery, stereotactic or external beam radiotherapy. In addition, numerous comorbidities were sought: cardiovascular or cerebrovascular disorders, diabetes, history of another cancer, chronic respiratory, renal, liver or pancreatic disease, inflammatory and systemic diseases requiring immunosuppressive or immunomodulating agents, neurological or psychiatric disorders, substance or alcohol abuse (**Supplementary Table 1**). The algorithm used to identify comorbidities in the SNDS database was based on the SNDS mapping tool, consisting in reference coding instructions developed to standardize the analysis of the morbidity burden from health-care utilization.[27,28] Long-term disease diagnoses, hospitalization discharge diagnoses and

medical procedures were sought in the 4 years prior to the anti-melanoma treatment initiation. In addition, medications were screened for in the 12 months before the anti-melanoma treatment initiation (4 years for certain immunosuppressive or immunomodulating agents).

Propensity score and pseudo-populations

Overlap weighting based on the propensity score was used to create pseudo-populations, in which characteristics measured were balanced between patients exposed and non-exposed to antibiotics.[29,30] A multivariate logistic regression was performed to calculate the propensity score for antibiotic exposure using all the covariates with non-zero values collected before the antibiotic timeframe. Treated patients were weighted by the probability of not receiving antibiotics (1 − propensity score) and untreated patients were weighted by the probability of receiving antibiotics (propensity score).[31,32] Covariate balance was checked using standardized differences in pseudo-populations in the exposed versus non-exposed groups.

Statistical analysis

Kaplan-Meier curves and weighted log-rank tests were performed within pseudo-populations. To evaluate the impact of antibiotic exposure on OS and TTD, a propensity score-weighted Cox proportional hazards regression model was used to estimate weighted hazard ratios (wHRs) and 95% confidence intervals (CIs). Proportional hazards were assessed by plotting log(-log(survival)) versus the log of survival time for categorical covariates, and scaled Schönfeld residuals versus survival time for continuous covariates. To study the prescription of antibiotics according to the progression of melanoma disease, the proportion of patients initiating an antibiotic course was plotted against time for patients receiving anti-PD-1 antibody or targeted therapy as a first treatment line. Because every individual living in France is included in the

SNDS database until death, there was no loss to follow-up. Statistical significance was defined at an a priori value of 0.05. Statistical analyses were performed using R v3.6.0 software (R Inc, USA). All statistical tests were 2-sided.

Results

Patient characteristics

In the anti-PD-1 antibody cohort (n=2605), 749 (28.6%) patients received antibiotics in the 3 months prior to anti-PD-1 initiation. In the targeted therapy cohort (n=1527), 460 (30.1%) patients received antibiotics in the 3 months prior to targeted therapy initiation. Age, sex, metastatic sites, previous surgery or radiotherapy and comorbidities were reported for both the ICI and targeted therapy cohorts. After overlap weighting, standardized mean differences between patients receiving or not receiving antibiotics were close to zero for all covariates (**Table 1**, **Supplementary Table 2**). All the characteristics measured were thus well balanced across groups within the pseudo-populations.

Antibiotic exposure before first-line anti-melanoma treatment

In the anti-PD-1 antibody cohort, 749 patients received 954 antibiotic prescriptions in the 3 months prior to their first-line treatment. In the targeted therapy cohort, there were 583 antibiotic prescriptions for 460 patients. The main antibiotics prescribed were amoxicillin/clavulanate (28.9%), amoxicillin (19.8%), pristinamycin (10.3%) and ofloxacin (4.5%). Primary infections requiring hospitalization were urinary tract (26.3%), respiratory tract (22.6%), skin and skin-associated structures infections (22.9%) and fever of undetermined cause (16.7%). The proportion of patients receiving an antibiotic prescription each day was estimated in the 2 years

before the initiation of the first-line anti-melanoma treatment. The incidence of antibiotic prescriptions rose steadily from 12 months before the first-line treatment in both cohorts (**Figure 1**).

Association between antibiotic use in the 3 months prior to anti-melanoma treatment and outcome

In the anti-PD-1 antibody cohort, 956 (36.7%) patients had died and 1602 (61.5%) had discontinued their first treatment line by December 31, 2017. Antibiotic exposure was not associated with shorter OS (wHR=1.01, 95% CI = 0.88-1.17) or TTD (wHR=1.00, 95% CI = 0.89-1.11). Kaplan-Meier curves were superimposed according to antibiotic receipt in the pseudo-populations (**Figure 2, A and B**).

In the targeted therapy cohort, 701 (45.9%) patients had died and 1074 (70.3%) had discontinued their first treatment line by December 31, 2017. Antibiotic exposure was not associated with OS (wHR=1.08, 95% CI = 0.92-1.27) or TTD (wHR=1.04, 95% CI = 0.91-1.18) (**Figure 2, C and D**).

Sensitivity analyses

As the impact of antibiotic treatment was suspected to be more detrimental in the month prior to ICI initiation, a first sensitivity analysis was performed narrowing the antibiotic timeframe to 1 month. No statistically significant association between antibiotic treatment and OS or TTD was evidenced in either cohort (**Figure 3**).

As high-impact antibiotics (fluoroquinolones, penicillins associated with β-lactamase inhibitors and second- and third-generation cephalosporins) induce more profound gut dysbiosis,[21–24] a second sensitivity analysis restricted the analysis to these antibiotics. In both the

anti-PD-1 antibody and targeted therapy cohorts, no statistically significant association between high-impact antibiotics and OS or TTD was evidenced (**Figure 3**).

In a third sensitivity analysis, patients hospitalized with a diagnosis of infection were excluded, as antibiotics administered during hospital stays are not recorded in the SNDS database. OS and TTD were not associated with antibiotic treatment after exclusion of these patients in either the anti-PD-1 antibody or the targeted therapy cohorts (**Figure 3**).

Crude HRs (resulting from analyses in the initial non-weighted populations) and wHRs (obtained from pseudo-populations) for the main and sensitivity analyses are provided in **Supplementary Table 3**.

Discussion

In this population-based study including 749 patients exposed to antibiotics compared to 1856 non-exposed patients, using an overlap weighting method based on a propensity score to balance characteristics of patients between groups, we evidenced that antibiotic use prior to anti-PD-1 antibody for advanced melanoma was not associated with worse outcome, whether for OS or for TTD. Consistent results were observed when the timeframe of antibiotic exposure was reduced from 3 to 1 month prior to anti-PD-1 initiation, or when exposure was restricted to antibiotics leading to more profound gut dysbiosis.

Considerable interest has been devoted to the investigation of how concomitant therapies with a potential immunomodulatory effect might interact with ICI among cancer patients. In particular, gut microbes could enhance antitumor immunity through T-cell responses to microbial antigens and cross-reactions with cancer antigens.[33,34] In keeping with this hypothesis, antibiotic

treatment could decrease ICI efficacy by altering the composition and diversity of the gut microbiota.[12,13,35–40] However, there is no consensus on which bacterial species are associated with response to anti-PD-1 antibody.[13,37,38] In addition, only a minority of the taxa forming the human gut microbiota are able to colonize mice, which makes extrapolating findings from rodent studies hazardous.[41,42] Thus, the link between the gut microbiota and response to ICI still needs to be further understood, and to date we lack experimental evidence on the issue of whether, how and for how long induced gut dysbiosis can alter ICI efficacy.[42]

In view of the immunological rationale, observational studies have investigated the effect of antibiotic exposure on ICI efficacy among cancer patients. The current body of knowledge derived from observational studies converges towards poorer prognosis among patients exposed to antibiotics in all cancers treated with ICI.[5,8–10,15,43,44] The association was often strong but the data were heterogeneous, mixing different treatment lines, different ICIs or even different types of cancer within the same studies. Relevant confounders could not be appropriately taken into account in most studies, due to small sample sizes and heterogeneity. For example, patients with certain comorbidities or locations of metastases could be more likely to receive antibiotics, and these data were absent or incomplete. This could also explain why the association between antibiotic exposure and poor prognosis was not related to the spectrum of the antibiotics used, nor therefore to their impact on the gut microbiota.[7,15] To disentangle the causal effect of antibiotic exposure from the confounding effect of patient characteristics, including patient fitness, comorbidities, and evolution of the metastatic disease, is a challenging issue.[14,15]

Confounding by indication is hard to fully capture with conventional methods, which inadequately control for prognostic variables.[16,45] However, confounding by indication can have a major impact on results. This bias can suggest an association that does not exist, or even reverse the direction of an association.[16] Regarding our population, we report in **Figure 1** the proportion

of patients receiving antibiotics, which increased with the approach of first-line anti-melanoma treatment. The steady increase in antibiotic use in the months preceding the initiation of the first-line treatment for metastatic melanoma could be in line with the intensification of care related to a symptomatic or complicated cancer. Antibiotic prescription could be associated with the discovery of the metastatic stage (*e.g.* pulmonary infections associated with lung metastases). In particular, a peak in antibiotic prescription was reached in the targeted therapy cohort in the month preceding the initiation of targeted therapy, which could suggest a strong confounding effect, and explain the statistically significantly increased crude HR for OS in the month prior to initiation in the targeted therapy cohort.

Our study has several strengths. First, while confounding is the alternative explanation for the shorter survival of patients receiving antibiotics in previous observational studies, the propensity score weighting method using overlap weights has been reported to show remarkable performance to avoid this bias.[30,46,47] Second, we identified a large number of comorbidities and provided precise information on metastatic locations using our database, which combines diagnosis-based and medication-based information with considerable historical depth.[17,28] These characteristics are relevant confounders in this context, and their identification enabled us to balance prognosis factors across groups. Third, the size of our nationwide database yielded a power of over 99% to demonstrate the previously suggested association among patients receiving anti-PD-1 antibody.[7,44] The number of antibiotic-exposed patients in our study was similar to the number in meta-analyses mixing studies on all cancer types, treatment lines and antibiotic timeframes.[6,8–10,43] In addition, the exhaustiveness of the database, with no loss to follow-up, protected from selection and attrition bias. Fourth, as there is no underlying rationale for antibiotics to impact the efficacy of targeted therapy, using the targeted therapy cohort as a negative control was an original and relevant approach to address our question. Fifth, TTD

corresponds to the time during which the disease is under control for a given treatment line, and was therefore used to reflect the impact of antibiotics on the first anti-melanoma treatment, irrespective of subsequent treatment lines.

Our study has limitations. First, prognostic factors like LDH levels, performance status or socioeconomic status were lacking in our database and could therefore not be used in the propensity score. Second, we used drug dispensations as a proxy for drug intake, but we could not ascertain the completion of antibiotic courses. However, even short antibiotic courses could have a prolonged effect on gut microbiota.[48,49] Third, antibiotics administered during hospital stays are not recorded in our database. Therefore, in a sensitivity analysis we excluded patients who had been hospitalized for infection during the timeframe of exposure, as they could have been misclassified. In addition, infections requiring hospitalization often correspond to severe infections,[50] and patients could have a competing risk of death, which justified excluding them. Fourth, participation in a clinical trial could not be identified in our database, because clinical trials entail no billing to the National Health Insurance. Fifth, strictly speaking, our results apply only to metastatic melanoma and one could be reluctant to extrapolate to other cancers. However, the two hypotheses discussed (antibiotic-induced gut dysbiosis impairing ICI efficacy and confounding by indication) concern general phenomena and should apply across other cancers treated with ICI.

Our results bring a robust contribution to the question of the impact of antibiotics on ICI efficacy and overall prognosis among cancer patients. Unlike previous findings, we show that antibiotic treatment before anti-PD-1 antibody initiation is not associated with decreased efficacy of anti-PD-1 antibody among metastatic melanoma patients. While some may consider that the jury is still out, we argue that strong and reasonable doubt exists for confounding by indication as the culprit accounting for a so-called antibiotic-induced detrimental effect on ICI efficacy. As a

consequence, from a clinical point of view, we suggest that physicians should not delay ICI for patients who have recently received antibiotics on the grounds of a risk of lack of efficacy. In addition, no data exist to substantiate what would be an appropriate and safe time-lag. Avoiding delaying immunotherapy is certainly a widely-shared attitude in metastatic settings, but it should also be recalled when administering ICI in adjuvant settings.

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Data Availability

The protocol and the statistical code are available on justified request. Under French law and regulations, databases extracted from the Systeme National des Données de Santé cannot be made available.

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Tables

Table 1. Characteristics of patients receiving anti-PD-1 antibody as a first-line treatment for metastatic melanoma and standardized mean differences according to antibiotic exposure

Characteristic	No. (%)	Standardized mean difference, %	
		In the initial population [a]	In the pseudo-population [b]
Antibiotics			
Antibiotics in the 3 months before initiation of the first-line treatment	749 (28.8)	-	-
Antibiotics in the month before initiation of the first-line treatment	336 (12.9)	-	-
High-impact antibiotics in the 3 months before initiation of the first-line treatment [a]	409 (15.7)	-	-
Age, years			
Mean (SD)	69 (14)	4.5	6.3×10^{-12}
Range	6-98		
Sex			
Male	1508 (57.9)	-3.6	-6.9×10^{-12}
Female	1097 (42.1))	-3.6	-6.9×10^{-12}
Location of metastatic sites [b]			
Brain	422 (16.2)	-11.6	4.9×10^{-12}
Lung	802 (30.8)	3.4	1.1×10^{-11}
Bone	273 (10.5)	9.8	2.1×10^{-11}
Liver	434 (16.7)	1.1	5.4×10^{-12}
Digestive system	242 (9.3)	-5.0	2.6×10^{-12}
Lymph node	1208 (46.4)	8.9	2.0×10^{-11}
Skin	504 (19.3)	-7.2	-7.2×10^{-12}
Mediastinum	24 (0.9)	0.2	3.4×10^{-12}
Urinary tract	133 (5.1)	-0.2	7.9×10^{-12}
Others	217 (8.3)	-5.1	-1.3×10^{-12}
Number of metastatic sites [b]			
Mean (sd)	1.6 (1.4)	-0.2	1.8×10^{-11}
Previous therapies [c]			
Stereotactic radiotherapy	129 (5.0)	-0.7	4.0×10^{-12}
External beam radiotherapy	134 (5.1)	0.6	5.0×10^{-13}
Lymphadenectomy	834 (32.0)	1.1	-4.2×10^{-12}
Surgical resection of distant metastases	309 (11.9)	-3.3	-4.6×10^{-12}
Comorbidities [d]			
Cardiovascular and cerebrovascular disease			
Recent acute ischemic heart disease [c]	29 (1.1)	1.2	3.7×10^{-12}
Chronic ischemic heart disease	235 (9.0)	2.9	7.2×10^{-12}

Recent acute ischemic cerebrovascular disease [c]	28 (1.1)	6.7	9.0×10^{-12}
Recent hemorrhagic stroke [c]	44 (1.7)	-4.0	4.7×10^{-12}
Sequelae or history of cerebrovascular disease	88 (3.4)	3.8	5.1×10^{-12}
Recent acute heart failure [c]	81 (3.1)	3.9	5.1×10^{-12}
Chronic heart failure	113 (4.3)	5.8	1.2×10^{-11}
Cardiac arrhythmia	284 (10.9)	3.2	4.7×10^{-12}
Cardiac valve disease	91 (3.5)	-0.2	1.7×10^{-12}
Recent acute peripheral vascular disease [c]	27 (1.0)	7.3	8.1×10^{-12}
Chronic peripheral vascular disease	181 (6.9)	0.0	-2.9×10^{-12}
Respiratory disease			
Chronic respiratory disease (including asthma and chronic obstructive pulmonary disease)	298 (11.4)	16.6	2.7×10^{-11}
Metabolic disease			
Diabetes	378 (14.5)	7.5	1.6×10^{-11}
Liver and pancreatic disease			
Mild or moderate liver disease	23 (0.9)	0.8	-5.4×10^{-13}
Severe liver disease	16 (0.6)	-1.5	-5.2×10^{-12}
Pancreatic disease	3 (0.1)	0.7	5.9×10^{-13}
Renal disease			
Chronic renal disease	168 (6.4)	6.5	1.2×10^{-11}
Dialysis [c]	5 (0.2)	2.3	3.6×10^{-12}
Kidney transplant [e]	3 (0.1)	0.7	2.7×10^{-12}
Immunosuppression			
HIV infection	5 (0.2)	2.3	3.6×10^{-12}
Solid organ transplant (except kidney) [e]	1 (0.04)	-	-
Inflammatory and systemic disease			
Inflammatory bowel disease	11 (0.4)	2.3	4.3×10^{-12}
Inflammatory rheumatic disorder or psoriasis requiring immunosuppressive or immunomodulating agents	22 (0.8)	7.0	8.1×10^{-12}
Connective tissue disease requiring immunosuppressive or immunomodulating agents	11 (0.4)	4.9	5.7×10^{-12}
Cancer			
Second cancer (other than melanoma)	410 (15.7)	-4.1	9.0×10^{-13}
Neurological disease			
Parkinson's disease and extrapyramidal syndromes	34 (1.3)	2.0	3.4×10^{-12}
Multiple sclerosis and related disorders	1 (0.04)	-	-
Paralysis	121 (4.6)	-9.1	1.0×10^{-11}
Neuromuscular disease	25 (1.0)	5.1	6.4×10^{-12}
Epilepsy	67 (2.6)	-2.7	4.8×10^{-12}
Dementia	42 (1.6)	-3.2	-2.7×10^{-12}
Mental deficiency	2 (0.1)	-	-
Psychiatric disease			
Hospitalization in a psychiatric hospital	33 (1.3)	0.9	-8.6×10^{-12}
Depression and mood disorders	425 (16.3)	9.9	1.7×10^{-11}

Schizophrenia and delusional disorders	55 (2.1)	-9.5	-5.3×10^{-11}
Alcohol abuse	81 (3.1)	-0.3	8.9×10^{-13}
Substance abuse	8 (0.3)	5.2	5.1×10^{-12}

[a] before overlap weighting

[b] after overlap weighting

- : covariates with zero values (no exposed or unexposed individual) were not included in the calculation of the propensity score.

Figure legends

Figure 1. Evolution of antibiotic use according to the progression of melanoma disease over time. The proportion of patients initiating an antibiotic course in the two years before the initiation of the first anti-cancer treatment line is shown for the anti-PD-1 cohort (red line) and the targeted therapy cohort (blue line). The 95% confidence band for the proportion of antibiotic prescriptions is shown in gray. The timeframe for antibiotics exposure in the main analysis is circled in orange.

Figure 2. Kaplan Meier curves for overall survival and time-to-treatment discontinuation according to antibiotic treatment in pseudo-populations of patients receiving anti-PD-1 antibody or targeted therapy as a first-line treatment for metastatic melanoma. Pseudo-populations are obtained using overlap weighting from the initial anti-PD-1 antibody cohort (A and B) and targeted therapy cohort (C and D). Patients receiving antibiotics in the 3 months prior to anti-PD-1 antibody or targeted therapy are represented by the blue line, non-exposed patients by the red line. Overall survival is shown in panels A and C, time-to-treatment discontinuation in panels B and D. Weighted log-rank tests are provided. The numbers of at-risk individuals correspond to fictive weighted individuals. All the study participants from the anti-PD-1 antibody cohort (n=2605) and the targeted therapy cohort (n=1527) were included. All statistical tests were 2-sided.

Figure 3. Forest plot summarizing the analyses performed in both cohorts of patients receiving anti-PD-1 antibody or targeted therapy as a first-line treatment for metastatic

melanoma. Main analysis investigated the impact of antibiotics in the 3 months prior to anti-melanoma treatment on overall survival and time-to-treatment discontinuation. In the first sensitivity analysis, the antibiotic timeframe was narrowed to 1 month prior to anti-melanoma treatment initiation. In the second sensitivity analysis, antibiotic exposure was restricted to antibiotics having a high impact on the gut microbiota (fluoroquinolones, second- and third-generation cephalosporins, and penicillins associated with β-lactamase inhibitors). In the third sensitivity analysis, patients with infections requiring hospitalization were excluded. The error bars represent the 95% confidence intervals (CIs). ATB = antibiotics ; HR = hazard ratio.

Figure 1-FINAL

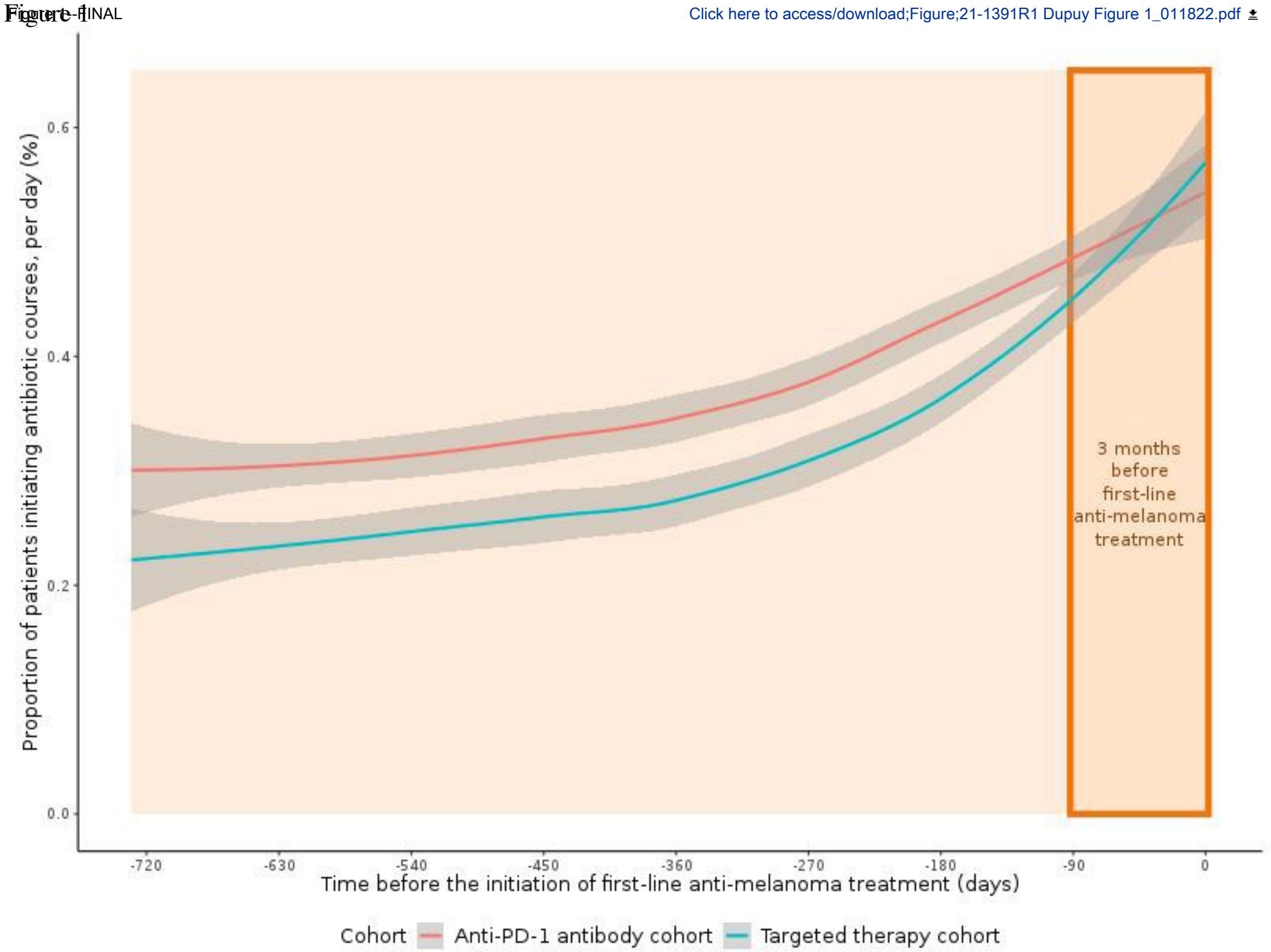

Figure 2_FINAL