Association of antibiotic exposure with survival and toxicity in patients with melanoma receiving immunotherapy

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Abstract

BACKGROUND:

Gut microbial diversity is associated with improved response to immune checkpoint inhibitors (ICI). Based on the known detrimental impact that antibiotics have on microbiome diversity, we hypothesized that antibiotic receipt prior to ICI would be associated with decreased survival.

METHODS:

Patients with stage III and IV melanoma treated with ICI between 2008 and 2019 were selected from an institutional database. A window of antibiotic receipt within 3 months prior to the first infusion of ICI was pre-specified. The primary outcome was overall survival (OS) and secondary outcomes were melanoma-specific mortality and immune-mediated colitis requiring intravenous (IV) steroids. All statistical tests were two-sided.

RESULTS:

There were 568 patients in our database, of which 114 received antibiotics prior to ICI. 35.9% of patients had stage III disease. On multivariable Cox proportional hazards analysis of patients with stage IV disease, the antibiotic-exposed group had statistically significantly worse OS (hazard ratio [HR] 1.81, 95% confidence interval [CI] 1.27-2.57, p<.001). The same effect was observed among antibiotic-exposed patients with stage III disease (HR 2.78, 95% CI 1.31-5.87, p=.007). When limited to only patients who received adjuvant ICI (N=89), antibiotic-exposed patients also had statistically significantly worse OS (HR 4.84, 95% CI 1.09-21.50, p=.04). The antibiotic group had a greater incidence of colitis (HR 2.14, 95% CI 1.02-4.52, p=.046).

CONCLUSION:

Patients with stage III and IV melanoma exposed to antibiotics prior to ICI had statistically significantly worse OS than unexposed patients. Antibiotic exposure was associated with greater incidence of moderate to severe immune-mediated colitis. Given the large number of antibiotics prescribed annually, physicians should be judicious with their use in cancer populations likely to receive ICI.

Immune checkpoint inhibitors (ICI) revolutionized the treatment of advanced melanoma, with clinical trials in the stage IV population showing long-term survival of 30-50% [1]. Despite these gains, a substantial proportion of patients do not respond to ICI, which has prompted intense interest in the discovery of predictive biomarkers to maximize the therapeutic benefit of ICI [2]. Animal and human studies show that the microbiome may be one such biomarker, with increased gut microbiome diversity and certain bacterial species being linked to improved ICI outcomes [3-6]. Additionally, it is known that antibiotic treatment has a profound effect on the microbiome, decreasing microbiome diversity and bacterial taxa known to have symbiotic functions in the gut [7-10]. Our study adds to prior literature by investigating the association of antibiotic use with both survival and ICI toxicity among patients with metastatic and non-metastatic melanoma.

Determining whether antibiotic-induced dysbiosis worsens ICI outcomes is essential given that in one large study, 30% of ambulatory antibiotic prescriptions were deemed inappropriate based on national guidelines [11]. We hypothesized that receipt of antibiotics within 3 months before the first infusion of ICI for patients with stage III and IV melanoma would be associated with worse overall survival (OS) and melanoma-specific mortality. Based on the microbiome's maintenance of immune tolerance in the local gut environment, we also hypothesized that dysbiosis would result in increased immune-mediated colitis [12-14].

Materials/Methods

Data Source and Patient Cohort

Patients with AJCC 8th edition stage III and stage IV melanoma treated with immune checkpoint inhibitors (ICI: ipilimumab, nivolumab, pembrolizumab, or ipilimumab/nivolumab) between 2008 and 2019 were selected from the University of Pennsylvania Abramson Cancer Center's melanoma research program protocol UPCC 08607 and IRB 703001 in accordance with the Institutional Review Board which waived written informed consent requirements [15]. Patients with prior treatment with targeted or cytotoxic systemic therapies were included. Patients who did not receive their first infusion of ICI at University of Pennsylvania, or who only received one dose of peri-operative antibiotics (e.g. intraoperative cefazolin before incision) were excluded.

Exposure and Outcome Variables

A time window of antibiotic receipt within 3 months (90 days) prior to the first infusion of ICI was pre-specified based on laboratory microbiome experiments showing protracted dysbiosis after exposure to antibiotics [7, 16]. Previous literature examining the effect of antibiotics on immunotherapy outcomes used time windows varying from 2 weeks to 3 months [17-22]. We did not include antibiotics received after ICI in order to avoid introducing immortal time bias [23]. Given the time-dependence of microbiota recovery after antibiotics, we also analyzed the data using a 1.5-month (45 day) window of antibiotic receipt. Receipt of antibiotics was ascertained from two sources: (1) structured data from the electronic health record (EHR) including prescriptions and infusions, and (2) physician note free text search using PennSeek, a custom tool that can search all free text in the EHR for large patient cohorts. All "hits" with EHR structured data and PennSeek were verified through manual chart review.

The primary outcome was OS and secondary outcomes were melanoma-specific mortality and immune-mediated colitis requiring IV steroids. Deaths were ascertained from the

institutional tumor registry, EHR death data, and internet obituaries. Death attribution was ascertained through the institutional tumor registry and manual chart review.

Stage III and stage IV populations were analyzed separately. We also measured the association between antibiotic exposure and survival in a "best-prognosis" subgroup by performing an analysis of Stage III patients receiving adjuvant ICI. As a sensitivity analysis, we measured the association between antibiotic exposure and survival in all patients after excluding patients who either received IV antibiotics or were hospitalized due to their infection. Finally, we measured the association between antibiotic exposure and immune-mediated colitis requiring IV steroids in the first year following initiation of ICI.

Covariates

Pre-specified covariates including age at the time of ICI, ECOG performance status, gender, race, AJCC 8th ed. stage at the time of ICI, relation of ICI to surgery (adjuvant, neoadjuvant, or no definitive surgery performed), prior targeted and cytotoxic therapies, BRAF status, LDH, ICI drug name, and infection data were ascertained from the University of Pennsylvania melanoma research program registry, the institutional tumor registry, and manual EHR review. Among patients with stage III disease, the reason for not undergoing a definitive resection was generally unresectable disease, such as a large regional recurrence.

Statistical Analyses

Kaplan-Meier and cumulative incidence curves were plotted to compare nominal OS (log-rank test) and melanoma-specific mortality (Gray's test) between groups. Cox proportional hazards models were used to estimate antibiotics' impact on OS while controlling for important confounders. These analyses were repeated using a 45-day window of antibiotic receipt before

the first infusion of ICI. The proportionality assumption was verified using Schoenfield residuals. As an exploratory analysis, we determined whether steroid receipt impacted OS by including IV steroid receipt (for colitis) as a time-varying covariate in the survival models. The impact of antibiotics on the incidence of colitis was determined with a Fine-Gray competing risk model controlling for the class of ICI received. Finally, we investigated the impact of individual classes of antibiotics on survival using Cox proportional hazards models. The primary analyses were performed in pre-specified subgroups (stage IV, stage III, adjuvant), while colitis, antibiotic class, and sensitivity analyses were performed on the entire sample for simplicity and greater statistical power. All analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC), and $p<.05$ was used as the threshold for statistical significance. All statistical tests were two-sided.

Results

Patient Characteristics

When analyzed by stage, the cohort of 568 patients (Figure 1) were balanced in the antibiotic-exposed (N=114) and -unexposed (N=454) groups with regards to age, gender, race, ECOG performance status, BRAF status, LDH, prior targeted therapy, prior chemotherapy, and ICI class received (Table 1). Among stage III patients, the antibiotic-exposed group had a greater proportion of patients who underwent a definitive surgical resection (93.4% vs. 65.8%). The reason is that patients undergoing surgery were more likely to receive post-operative antibiotics. Of patients who received antibiotics, 20.1% received an IV antibiotic with or without an oral antibiotic, 12.3% were hospitalized due to the infection resulting in antibiotic receipt, and no

patients died of the infection. Fifty-three percent of infections prompting antibiotics were soft tissue—commonly post-operative wound infection or for enlarged lymph nodes at diagnosis (Table 2). All other presenting problems constituted less than 10% of antibiotics-exposed patients. The most common antibiotic class received was cephalosporin (42.1% of exposed patients), followed by 24.6% penicillin and 21.1% fluoroquinolone (Supplementary Table 1). The mean time between initiation of antibiotics and the first infusion of ICI was 40 days.

Survival Outcomes

Median follow-up was 3.1 years and 60.6% of patients were alive at last follow-up; 37.9% died due to melanoma, 1.3% died due to a non-melanoma cause, and 1 patient (0.1%) died of an unknown cause. Among all patients, median survival was statistically significantly longer among the antibiotic-unexposed patients compared to antibiotic-exposed patients (Figure 2A: 43.7 vs. 27.4 months, log-rank p=.01). The cumulative incidence of melanoma-specific mortality was also greater among antibiotic-exposed patients (Figure 2B: 2-year cumulative incidence of 37.4% for unexposed vs. 47.0% for exposed, Gray's test p=.03). The survival detriment of antibiotic exposure was present on multivariable Cox proportional hazards analysis controlling for ICI class, race, stage (AJCC 8th ed. Stage III or IV), ECOG performance status, relation of ICI to surgery (adjuvant, neoadjuvant, or no definitive surgery performed), gender, and age (Table 3: hazard ratio [HR] 1.95, 95% confidence interval [CI] 1.43-2.66).

Among patients with stage IV melanoma, on multivariable Cox proportional hazards analysis controlling for age, stage (AJCC 8th ed. M stage), ECOG performance status, prior targeted therapy, gender, ICI class, race, BRAF status, and LDH, the antibiotic-exposed group experienced statistically significantly worse OS compared to the unexposed patients (Table 3: HR 1.81, 95% CI 1.27-2.57).

Among patients with stage III melanoma, on multivariable Cox proportional hazards analysis controlling for age, stage (AJCC 8th ed. sub-stages), gender, ICI class, and whether a definitive operation was performed (yes vs. no), the exposed group experienced statistically significantly worse OS than the unexposed group (Table 3: HR 2.78, 95% CI 1.31-5.87). On multivariable Cox proportional hazards regression analysis in the adjuvant ICI subgroup, antibiotic exposure was associated with statistically significantly worse OS (Table 3: HR 4.84, 95% CI 1.09-21.50).

Given the variety of time windows for antibiotic receipt reported in the literature, we evaluated the robustness of our findings by repeating our analyses using a window of antibiotic receipt of 45 days rather than 90 days. Among the stage III and IV patients, antibiotic receipt was still associated with statistically significantly worse OS (Figure 3: stage III: HR 3.74, 95% CI 1.72-8.14; stage IV: HR 1.79, 95% CI 1.19-2.69).

We performed a sensitivity analysis in which we excluded patients who either received IV antibiotics or were hospitalized due to the infection that led to antibiotics being prescribed. In this group with only the most minor infections, on multivariable analysis, antibiotic exposure was still statistically significantly associated with worse survival (Table 3: HR 1.89, 95% CI 1.32-2.71). The forest plot in Figure 3 summarizes the multivariable hazard ratios for antibiotic exposure across all analyses.

We next examined the hazard associated with individual classes of antibiotics and found a survival detriment for penicillins (N=28, HR 2.63, 95% CI 1.55-4.48), cephalosporins (N=48, HR 1.70, 95% CI 1.07-2.71), and fluoroquinolones (N=24, HR 1.67, 95% CI 1.00-2.80), as seen in Supplementary Table 1. Other categories—vancomycin (N=7), macrolides/tetracyclines

(N=16), TMP/SMX (N=12), clindamycin (N=10), and metronidazole (N=7)—were not associated with worse survival but also did have much smaller numbers of exposed patients.

Immune-mediated Colitis

The 1-year cumulative incidence of immune-mediated colitis requiring IV steroids was statistically significantly higher among antibiotic-exposed patients compared to unexposed (Supplementary Figure 1: 1-year cumulative incidence 9.8% vs. 4.6%, p=.03). In a Fine-Grey multivariable competing risk regression model controlling for ICI class, antibiotic exposure was associated with statistically significantly more colitis requiring IV steroids (Table 4: HR 2.14, 95% CI 1.02-4.52). Despite the potential link between antibiotics and *C. difficile* colitis, we identified only 3 total patients with confirmed *C. difficile* colitis in the one year following initiation of ICI.

In order to explore whether the connection between antibiotic exposure and decreased survival was mediated by increased steroid use, we performed a survival analysis among all patients with IV steroid receipt (to treat colitis) as a time-varying covariate. Receipt of antibiotics remained a statistically significant predictor of worse OS (HR 1.47, 95% CI 1.10-1.97).

Discussion

The gut microbiome is emerging as a predictive biomarker for the efficacy of ICI against advanced melanoma. A knowledge gap exists as to what impact antibiotics have on the efficacy of ICI given the profound effects they exert on the microbiome and the well-described interaction between the microbiome and the immune system. Our institutional study is the first to stratify patients by stage and examine rates of immune-mediated colitis. Using a database of 568

patients, we discovered that patients exposed to antibiotics within the 3 months prior to their first infusion of ICI had statistically significantly worse OS and melanoma-specific mortality than unexposed patients. Importantly, this association was seen in both the stage III and stage IV populations, and when limiting the analysis to the adjuvant ICI subgroup—the patients with the best expected prognosis—we still noted a statistically significant survival difference. The survival detriment with antibiotics was present even on sensitivity analysis excluding patients who received IV antibiotics or were hospitalized for the infection that prompted the antibiotics. Our primary finding proved robust to time interval definition, as we still observed statistically significant survival differences when defining antibiotic exposure as receipt within the 45 days prior to the first ICI infusion. Finally, we found that the cumulative incidence of immune-mediated colitis requiring IV steroids was statistically significantly higher among antibiotics-exposed patients, supporting the hypothesis that these patients have clinically significant dysbiosis.

The hypothesis—that antibiotic exposure is associated with worse ICI outcomes in melanoma—is built upon multiple animal and human studies showing, 1) higher microbiome diversity and certain commensal bacteria are associated with an improved response to ICI, and 2) antibiotic treatment causes profound dysbiosis and may be associated with worse ICI outcomes. Initial research studying the interaction of gut microbiota and ICI efficacy was performed in mouse models and demonstrated that *Bacteroides* and *Bifidobacterium* species were necessary for ICI efficacy, and that oral gavage of *Bacteroides* species was sufficient to restore anti-cancer efficacy of ICI in germ-free mice [6, 24]. Chaput et al. subsequently showed in a cohort of 26 melanoma patients treated with ipilimumab that subjects with microbiomes enriched with *Faecalibacterium* had longer progression-free survival (PFS) and OS [25]. A trio of studies

published in *Science* in 2018 showed that responders to ICI had characteristic microbiota signatures, and observed a cause-effect relationship between the microbiome and response to ICI through fecal microbiota transplant of gut bacteria from human ICI responders to germ-free mice. Concurrent with studies examining the link between the microbiome and the immune system, modern genomic sequencing technologies have shown that antibiotic treatment profoundly affects the microbiome by decreasing microbial diversity, increasing populations of pathogenic species, and causing population shifts that can take up to 12 months to recover to baseline [7-10, 26, 27].

Consistent with the immune anti-cancer benefits of a favorable microbiome and the disruptive effect of antibiotics, our study showed that patients with stage III and IV melanoma exposed to antibiotics prior to ICI had statistically significantly worse survival than unexposed patients. Previous clinical literature has been consistent in showing negative impacts of antibiotic exposure on ICI outcomes. Routy et al. studied a cohort of 249 patients with non-small cell lung cancer (NSCLC), renal cell carcinoma, or urothelial carcinoma and observed statistically significantly worse OS and PFS among patients who received antibiotics within 2 months before, or 1 month after the first infusion of ICI [3]. Multiple other published studies have shown in various histologies that antibiotic-exposed patients have worse ICI outcomes than unexposed patients, with the exception of one study in NSCLC patients that found no association (although other studies did show a survival detriment among NSCLC patients) [17, 18, 21, 22, 28-30].

A unique feature of our study is that we performed key subgroup and sensitivity analyses to determine the robustness of the survival difference we observed in the primary analysis. A novel finding in our study is that patients with non-metastatic melanoma receiving ICI in the adjuvant setting also have a survival detriment if exposed to antibiotics. In fact, we noted larger survival

differences as cancer prognosis improved. The multivariable hazard ratios for death for the stage IV, all stage III, and stage III adjuvant ICI subgroups were 1.81 (p=.001), 2.78 (p=.007), and 4.84 (p=.04), respectively. The directionality of survival detriment, with better-prognosis patients experiencing greater harm from antibiotics, suggests that antibiotic-induced dysbiosis could mean the difference between long-term cure and metastatic recurrence in high-risk Stage III patients. Clearly these findings warrant validation studies with other large data sets.

The large sample size of our study also allowed us to explore the impact of individual antibiotic classes and drugs on OS. We observed that penicillins, cephalosporins, and fluoroquinolones were associated with worse OS on multivariable analysis. Studies analyzing the impact of specific classes of antibiotics on microbiota preceded the next-generation sequencing era, but interestingly found with culture techniques that penicillins, cephalosporins, and fluoroquinolones decrease populations of *Bifidobacteria* and *Lactobacilli*, two taxa thought to be important to the beneficial functions of the microbiome [26, 31]. While antibiotic classes would be expected to affect microbiome composition differently, it is not yet known whether antibiotic classes impact the microbiome function differently as well.

Another novel finding of our study is that antibiotic-exposed patients experienced a higher rate of immune-mediated colitis requiring IV steroids within the 1 year following the initiation of ICI (HR 2.14, 95% CI 1.02-4.52). This finding may seem counterintuitive given our observation of decreased ICI efficacy in the setting of antibiotic use, but actually is in keeping with the microbiome's role in maintaining immune tolerance to non-pathogenic antigens; indeed, dysbiosis is linked to multiple systemic and intestinal inflammatory disorders [12-14]. The physiology of the microbiome-immune axis supports a hypothesis of antibiotics being linked to both decreased efficacy of ICI and increased immune-mediated colitis. ICI-induced colitis is still

poorly understood, but its mechanism may depend on inhibition of mucosal regulatory T cells, which are induced by the normal microbiome [12, 32, 33]. Additionally, regrowth of pro-inflammation bacterial species after antibiotics may act synergistically with ICI mechanisms to promote colitis. The higher rate of colitis raises the question of whether the decreased OS in the antibiotic-exposed group could be due to greater steroid exposure. Studies are consistent in showing that patients who start a steroid for an ICI toxicity do not appear to have decreased ICI efficacy [34, 35]. We nevertheless explored this potential mechanism in our own data by including steroid receipt as a time-varying covariate and found that steroids did not impact OS and did not change the association of antibiotic exposure with decreased OS.

There are several limitations of our study. The non-randomized, retrospective study design makes it impossible to control for unknown confounders. While the study design is a limitation, a randomized trial testing this hypothesis would not be ethical and will likely never be done. An important future direction that would address our study's limitations would be a prospective cohort study that carefully records antibiotic receipt, important confounders, and collects biosamples for biomarker discovery and mechanistic exploration.

In conclusion, we demonstrate that antibiotic exposure before initiation of ICI is associated with statistically significantly worse survival in both stage III and stage IV melanoma patients, and is also associated with greater moderate to severe immune-mediated colitis. This finding supports our hypothesis and is consistent with multiple animal and human studies showing that dysbiosis is linked to worse ICI efficacy. Given the known overutilization of antibiotics in current society, physicians should exercise caution when considering antibiotic prescription in cancer populations treated with ICI [11]. These findings are particularly relevant

for patients undergoing surgery for stage III or IV melanoma, in whom adjuvant ICI therapy is planned, as injudicious antibiotic use in the post-operative setting should be avoided if possible.

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Table 1: Baseline characteristics stratified by stage and antibiotic receipt status

Received antibiotics within 90 days	All No. (%)	Antibiotics No. (%)	No antibiotics No. (%)
STAGE IV, No.	364	68	296
Age at time of first immunotherapy infusion			
Median (Range)	64.0 (18.0 - 94.0)	62.5 (33.0 - 89.0)	65.0 (18.0 - 94.0)
Mean (SD)	62.6 (13.6)	61.8 (13.0)	62.8 (13.7)
IQR	55.0 - 72.0	55.0 - 70.0	54.5 - 72.0
Consolidated racial categories			
Black	8 (2.2)	4 (5.9)	4 (1.4)
Other or unknown race	30 (8.2)	6 (8.8)	24 (8.1)
White	326 (89.6)	58 (85.3)	268 (90.5)
Gender			
Female	121 (33.2)	22 (32.4)	99 (33.4)
Male	243 (66.8)	46 (67.6)	197 (66.6)
ECOG Performance Status			
0	185 (50.8)	32 (47.1)	153 (51.7)
1	138 (37.9)	28 (41.2)	110 (37.2)
2	16 (4.4)	2 (2.9)	14 (4.7)
3	6 (1.6)	3 (4.4)	3 (1.0)
Missing	19 (5.2)	3 (4.4)	16 (5.4)
LDH			

Greater than the upper limit of normal	148 (40.7)	26 (38.2)	122 (41.2)
Unknown	36 (9.9)	10 (14.7)	26 (8.8)
Within normal limits	180 (49.5)	32 (47.1)	148 (50.0)
BRAF mutation status			
BRAF mutant	142 (39.0)	22 (32.4)	120 (40.5)
No BRAF mutation	222 (61.0)	46 (67.6)	176 (59.5)
AJCC 8th ed Stage			
M1a	41 (11.3)	9 (13.2)	32 (10.8)
M1b	81 (22.3)	11 (16.2)	70 (23.6)
M1c	157 (43.1)	29 (42.6)	128 (43.2)
M1d	85 (23.4)	19 (27.9)	66 (22.3)
Targeted therapy received before initial immunotherapy?			
No	294 (80.8)	54 (79.4)	240 (81.1)
Yes	70 (19.2)	14 (20.6)	56 (18.9)
Chemotherapy received before initial immunotherapy?			
No	331 (90.9)	65 (95.6)	266 (89.9)
Yes	33 (9.1)	3 (4.4)	30 (10.1)
Class of immunotherapy received			
Anti-CTLA4	158 (43.4)	25 (36.8)	133 (44.9)
Anti-PD1	160 (44.0)	30 (44.1)	130 (43.9)
Combined anti-CTLA4 and anti-PD1	46 (12.6)	13 (19.1)	33 (11.1)

Received antibiotics within 90 days	All No. (%)	Antibiotics No. (%)	No antibiotics No. (%)
STAGE III, No.	204	46	158
Age at time of first immunotherapy infusion			
Median (Range)	62.0 (18.0 - 85.0)	60.0 (18.0 - 85.0)	63.0 (19.0 - 85.0)
Mean (SD)	59.7 (15.7)	58.7 (14.5)	60.0 (16.0)
IQR	49.5 - 72.0	50.0 - 70.0	49.0 - 73.0
Race			
Black	0 (0)	0 (0)	0 (0)
Other or unknown race	23 (11.3)	6 (13.0)	17 (10.8)
White	181 (88.7)	40 (87.0)	141 (89.2)
Gender			
Female	76 (37.3)	14 (30.4)	62 (39.2)
Male	128 (62.7)	32 (69.6)	96 (60.8)
ECOG Performance Status			
0	158 (77.5)	36 (78.3)	122 (77.2)
1	39 (19.1)	10 (21.7)	29 (18.4)
2	2 (1.0)	0 (0)	2 (1.3)
Missing	5 (2.5)	0 (0)	5 (3.1)
BRAF mutation status			
BRAF mutant	71 (34.8)	16 (34.8)	55 (34.8)
No BRAF mutation	82 (40.2)	22 (47.8)	60 (38.0)

Unknown	51 (25.0)	8 (17.4)	43 (27.2)
AJCC 8th ed Stage			
Stage IIIA	27 (13.2)	4 (8.7)	23 (14.6)
Stage IIIB	58 (28.4)	15 (32.6)	43 (27.2)
Stage IIIC	113 (55.4)	26 (56.5)	87 (55.1)
Stage IIID	6 (2.9)	1 (2.2)	5 (3.2)
Class of immunotherapy received			
Anti-CTLA4	74 (36.3)	17 (37.0)	57 (36.1)
Anti-PD1	126 (61.8)	29 (63.0)	97 (61.4)
Combined anti-CTLA4 and anti-PD1	4 (2.0)	0 (0.0)	4 (2.5)
Relation of immunotherapy to definitive surgery			
Adjuvant	89 (43.6)	33 (71.7)	56 (35.4)
Neoadjuvant	58 (28.4)	10 (21.7)	48 (30.4)
No definitive surgery performed	57 (27.9)	3 (6.5)	54 (34.2)

Table 2: Suspected or confirmed infections leading to receipt of antibiotic

Type of infection	Stage IV (%)	Stage III (%)	Total
Soft tissue	24 (35.3)	36 (78.3)	60
Pneumonia	10 (14.7)	0 (0.0)	10
Bronchitis	6 (8.8)	0 (0.0)	6
Dental	4 (5.9)	1 (2.2)	5
Sinus	4 (5.9)	1 (2.2)	5
Urinary	3 (4.4)	2 (4.3)	5
Fever of unknown source	2 (2.9)	0 (0.0)	2
Gastrointestinal	2 (2.9)	0 (0.0)	2
Sepsis	2 (2.9)	0 (0.0)	2
Bacteremia	1 (1.5)	0 (0.0)	1
Other	3 (4.4)	2 (4.3)	5
Unknown	7 (10.3)	4 (8.7)	11

Table 3: Multivariable Cox proportional hazards models for stage IV, stage III, and adjuvant patient groups as well as sensitivity analysis excluding infections requiring IV antibiotics or hospitalization

Subgroup model	Antibiotic receipt multivariable* hazard ratio for OS (95% confidence interval)	P value
Model 1: All patients	1.95 (1.43-2.66)	<.001
Model 2: Stage IV	1.81 (1.27-2.57)	.001
Model 3: All stage III	2.78 (1.31-5.87)	.007
Model 4: Only adjuvant	4.84 (1.09-21.50)	.04
Model 5: Sensitivity analysis	1.89 (1.32-2.71)	<.001

*Covariates for: 1) All patients: ICI class, race, stage, ECOG, relation of ICI to surgery (adjuvant, neoadjuvant, or no definitive surgery performed), gender, and age. 2) Stage IV: ICI class, race, stage, ECOG, gender, age, prior targeted therapy, LDH, and BRAF mutation status. 3) All stage III: ICI class, race, stage, gender, age, relation of ICI to surgery. 4) Only adjuvant: ICI class, race, gender, age. 5) Sensitivity analysis: ICI class, race, stage, ECOG, relation of ICI to surgery, gender, age. Full model output in Supplementary Table 2.

Table 4: Multivariable Fine-Grey competing risk model for association between antibiotic receipt and immune-mediated colitis requiring IV steroids in the first 1 year following ICI for all patients

Variable	Multivariable hazard ratio for colitis (95% confidence interval)	P value
Received antibiotics		
Yes	2.14 (1.02-4.52)	.046
No	1.00 (Ref)	
ICI class		
Anti-CTLA4 + Anti-PD1	3.42 (1.37-8.57)	.009
Anti-PD1	0.74 (0.33-1.69)	.47
Anti-CTLA4	1.00 (Ref)	

Figure 1: CONSORT diagram

Figure 2: Overall survival and melanoma-specific mortality outcomes for all patients stratified by antibiotic receipt. The log-rank test was performed for the overall survival curves and Gray's test for melanoma-specific mortality. All statistical tests were two-sided. Solid lines represent patients not exposed to antibiotics in a 3-month time window and dashed lines represent patients who were exposed. A) Kaplan-Meier survival plot of overall survival stratified by antibiotic receipt for all patients with a number at risk table; B) Cumulative incidence of melanoma-specific mortality with non-melanoma death as a competing risk, stratified by antibiotic receipt for all patients. Abx, antibiotics.

Figure 3: Forest plot of multivariable hazard ratios associating overall survival with antibiotic receipt among multiple patient groups with both a 3-month and 1.5-month time window for antibiotic receipt. Error bars represent the 95% confidence interval bounds. HR, hazard ratio; CI, confidence interval.

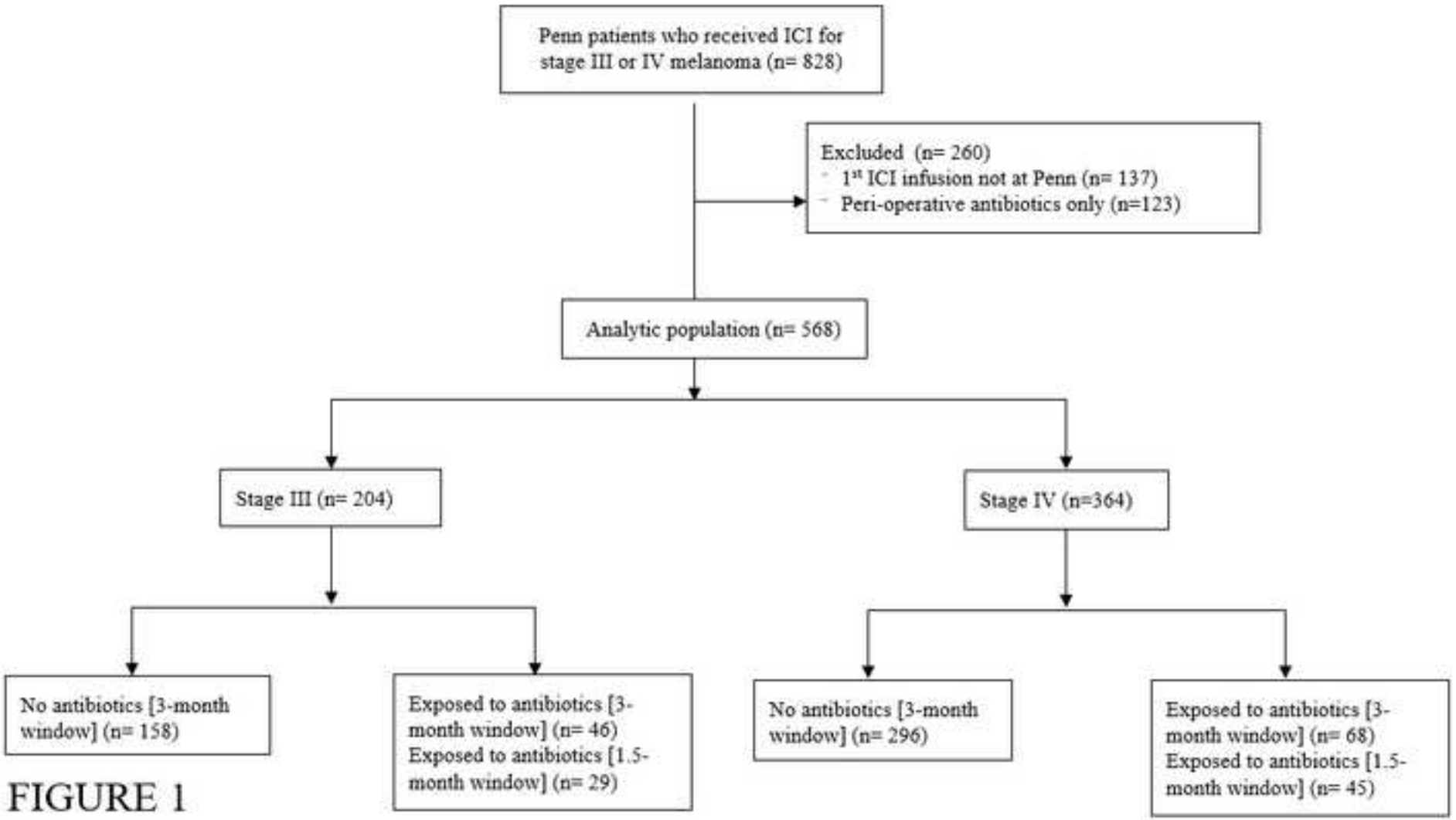
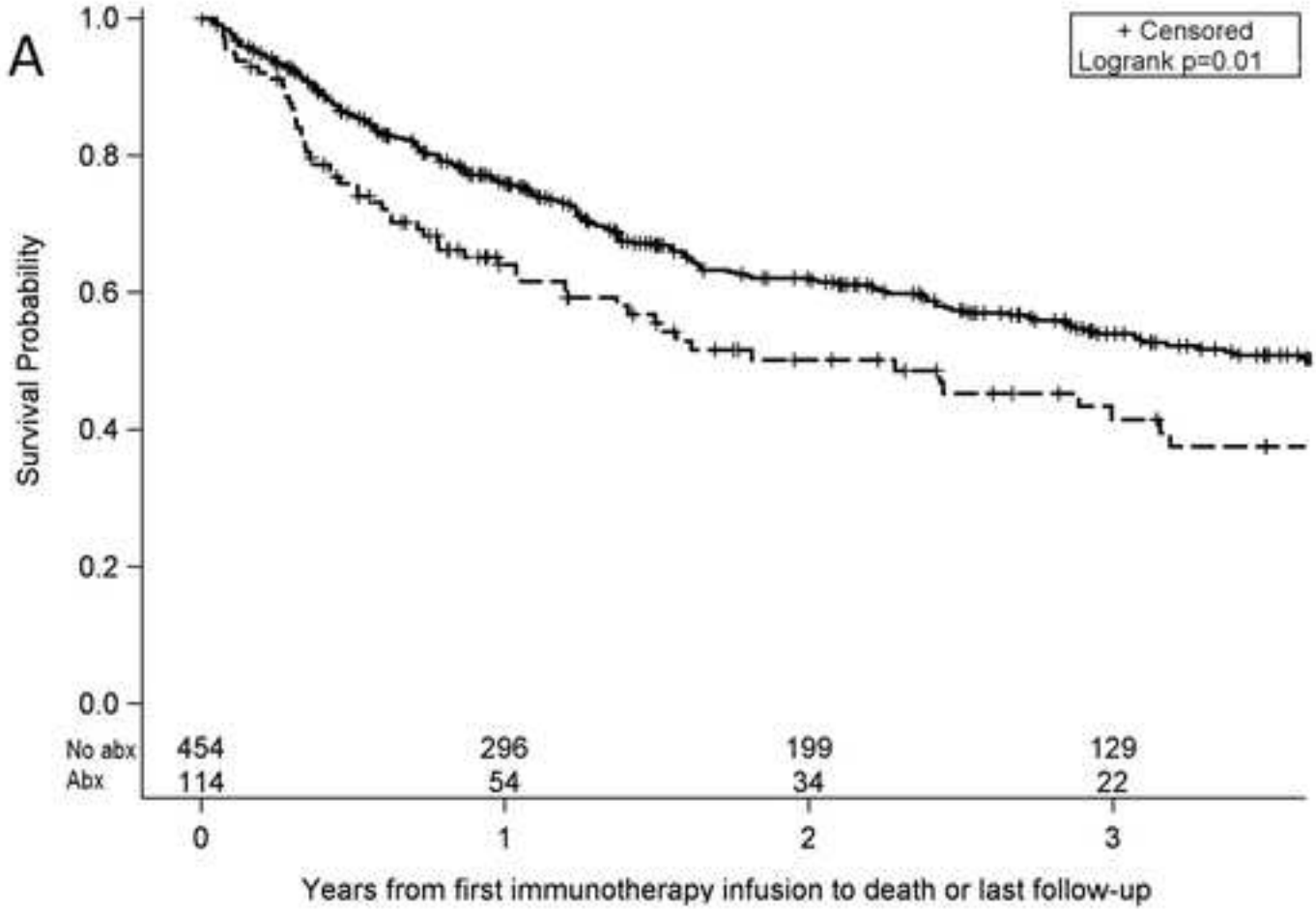


FIGURE 1

Figure 2--FINAL



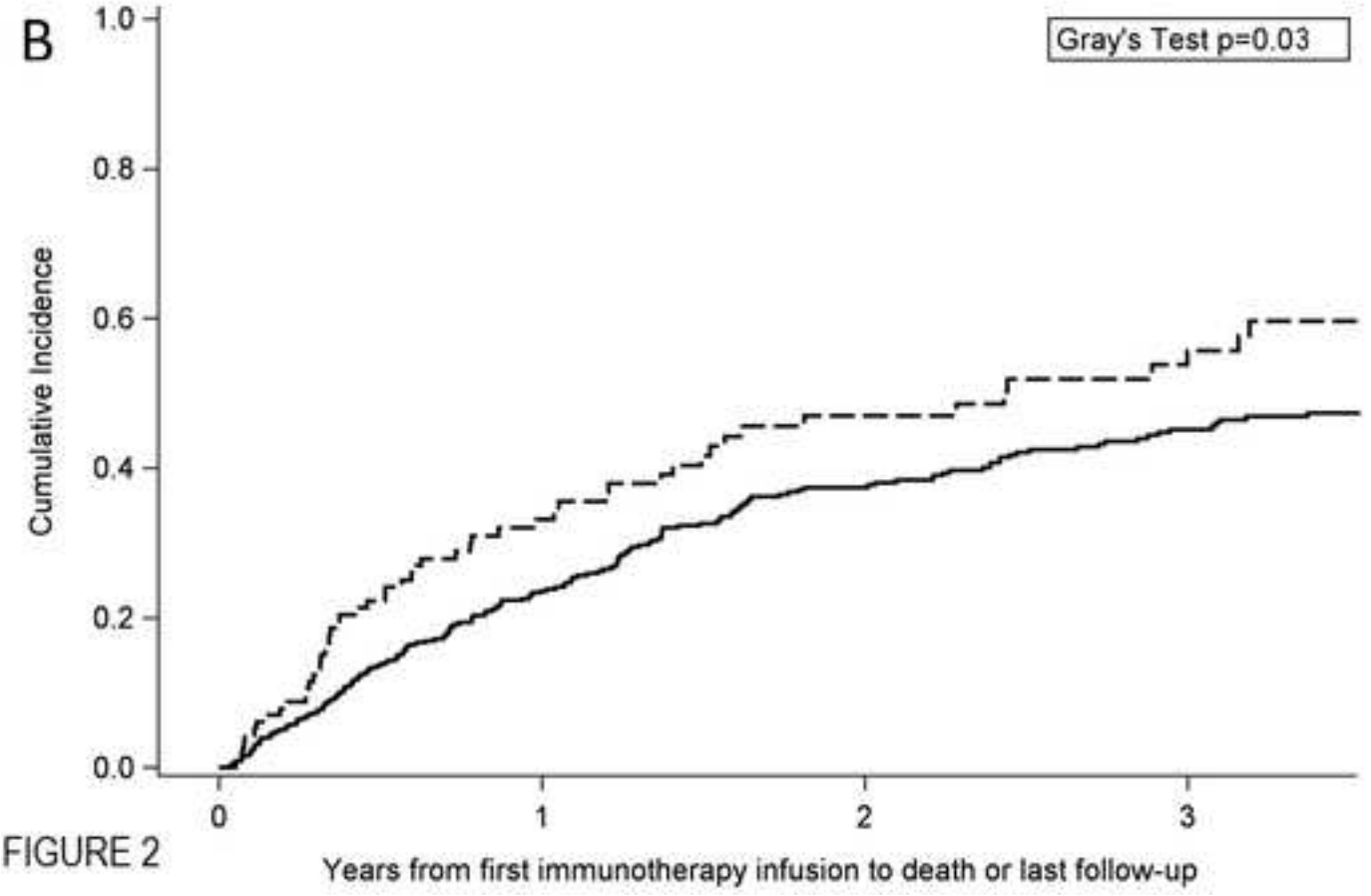


FIGURE 2

Figure 3--FINAL

3_040720.png

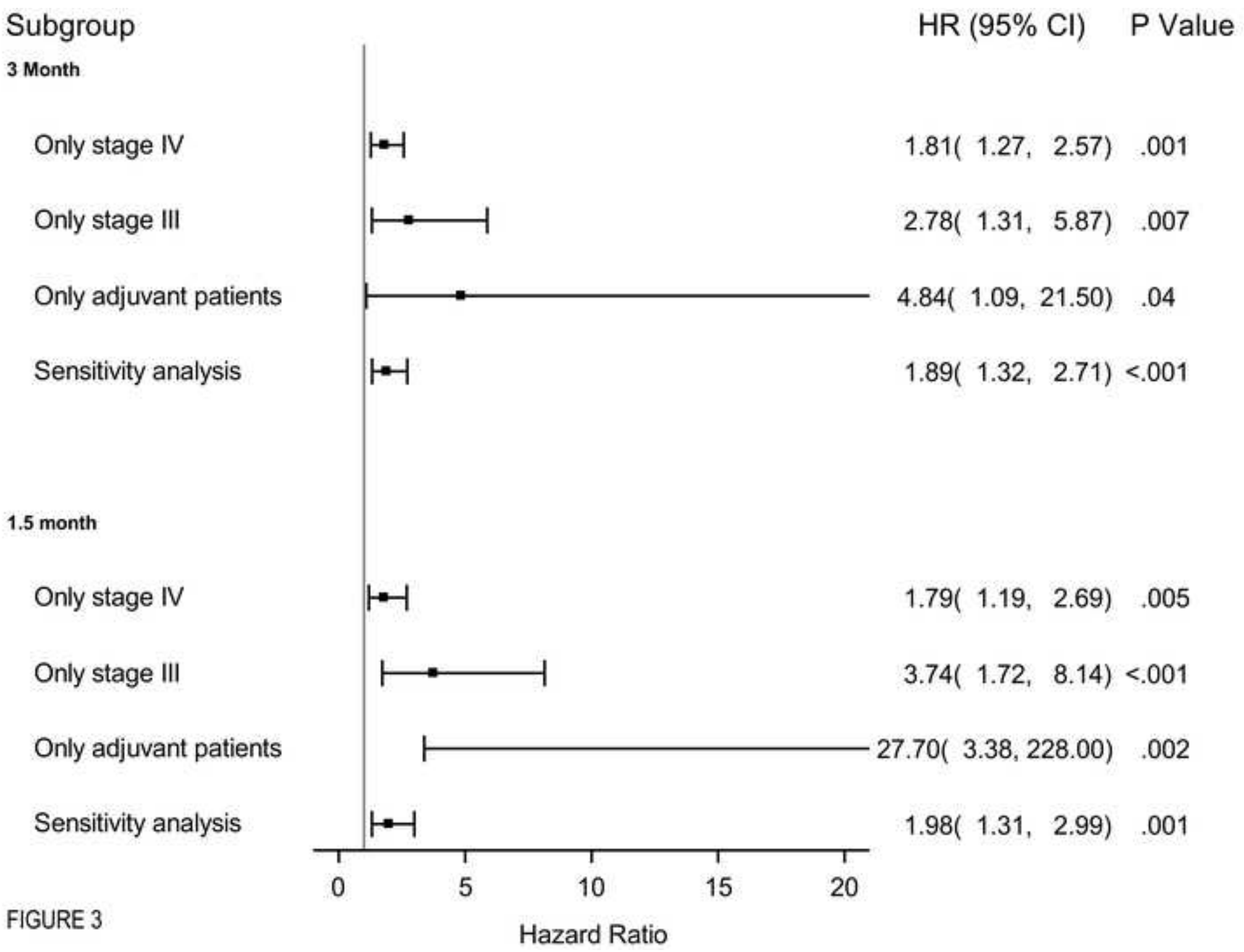


FIGURE 3