Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors

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Background: The gut microbiota has been shown to be an important determinant of the efficacy of immune checkpoint inhibitors (ICIs) in cancer. Several lines of evidence suggest that antibiotic (ATB) usage prior to or within the first month of ICI initiation negatively impacts clinical outcomes.

Methods: We examined patients with advanced melanoma treated with an anti-PD-1 monoclonal antibody (mAb) or an anti-CTLA-4 mAb alone or in combination with chemotherapy. Those receiving ATB within 30 days of beginning ICI were compared with those who did not receive ATB. Response rates as determined by RECIST 1.1, progression-free survival (PFS), overall survival (OS) and immune-related toxicities were assessed.

Results: Of these 74 patients analyzed, a total of 10 patients received ATB (13.5%) within 30 days of initiation of ICI. Patients who received ATB 30 days prior to the administration of ICI experienced more primary resistance (progressive disease) (0% of the objective response rate compared to 34%), and progression-free survival (PFS) was significantly shorter (2.4 vs 7.3 months, HR 0.28, 95% CI (0.10–0.76) \( p = 0.01 \)). Overall survival (OS) was also shorter; however, this was not statistically significant (10.7 vs 18.3 months, HR 0.52, 95% CI (0.21–1.32) \( p = 0.17 \)). The multivariate analysis further supported that ATB administration was associated with worse PFS (HR 0.32 (0.13–0.83) 95% CI, \( p = 0.02 \)).

Conclusion: These findings suggest that ATB use within 30 days prior to ICI initiation in patients with advanced melanoma may adversely affect patient outcomes.

Introduction

Immune checkpoint inhibition has revolutionized the therapeutic landscape of advanced melanoma and now represents the standard of care. Indeed, ipilimumab, an anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) monoclonal antibody (mAb), was the first immune checkpoint inhibitor (ICI) to be approved in the metastatic setting.\(^1\,\,^2\) Subsequently, mAbs pembrolizumab and nivolumab, which inhibit programmed cell death protein-1 (PD-1), have been associated with improved overall survival (OS), progression-free survival (PFS) and lower immune-mediated toxicities in comparison to ipilimumab alone.\(^3\,\,^4\) Combinations of ICIs provide deeper response rates; however, toxicities remain the principal hurdle.\(^5\) Now, the use of ICIs in both neoadjuvant and adjuvant settings represent a promising therapeutic avenue for patients with high-risk locally advanced melanoma.\(^6\,\,^7\)

The clinical trials described above also showed that the incidence of primary resistance to immune checkpoint inhibitors (ICI) ranges from 40% to 65% with anti-PD-1 and >40% with anti-PD-1 in combination with anti-CTLA-4 therapy. As a result, major efforts are ongoing to identify biomarkers that predict response to ICI as well as new strategies to improve ICI efficacy and clinical outcomes.

Several lines of evidence suggest that antibiotic (ATB) usage prior to or within the first month of ICI initiation negatively impacts the clinical outcomes in non-small cell lung cancer (NSCLC), renal cell cancer (RCC), and urothelial cancer (UC).\(^8\) Furthermore, the gut microbiota has been shown to be a key determinant in the immune-cancer set point with specific commensals such as Ruminococcus, Bifidobacterium indistinctus and Akkermansia muciniphila having particularly important roles in melanoma, NSCLC, and RCC. Patients enriched with these commensals had improved clinical responses which correlated with a stronger T lymphocyte CD8\(^+\) response.\(^11\,\,^12\)

Given the clinical evidence of the detrimental impact of ATB-induced dysbiosis and the clinical correlation with gut microbiota composition, we sought to determine if the use of...
ATB prior to the initiation of ICI was associated with worse clinical outcomes in patients with advanced melanoma.

**Results**

A total of 74 patients from two academic centers with stage IV melanoma were identified. Of these 74 patients, a total of 10 patients received ATB (13.5%) within 30 days of ICI initiation. In this study, the majority of patients received single-agent ICI as first-line therapy. Of the patients that received ICI alone, 41 (55.4%) received pembrolizumab, 13 received (17.6%) nivolumab, and 5 (6.8%) received ipilimumab. Fifteen (20.3%) patients received a combination of ipilimumab plus carboplatin/paclitaxel as part of a clinical trial (Table 1). β-lactams ± inhibitors were the most commonly administered ATB. Antibiotics were administered both orally (40%) and intravenously (60%) for a wide range of indications, and the majority of patients received ATB for a duration of over 7 days (Supplemental Table 1). The patient demographics of the ATB and non-ATB groups are delineated in (Table 1). The mean age of the studied population was 65, with most patients having an ECOG performance status of 0 or 1. Upon progression, the majority of patients (100% in the ATB group and 82.8% in non-ATB group) did not receive further treatment.

In patients who received ATB 30 days prior to the administration of ICI, PFS was significantly shorter (2.4 vs 7.3 months, HR 0.28, 95% CI (0.10–0.76) p = 0.01). Overall survival was also shorter; however, this was not statistically significant (10.7 vs 18.3 months, HR:0.52, 95% CI (0.21–1.32) p = 0.17) (Figure 1(a–b)). When considering patients who received ICI alone (n = 59) without chemotherapy (a more realistic representation of current clinical practice), OS was significantly shorter in patients with ATB exposure (n = 7) (OS 7.5 vs 18.3 months, HR 0.27 95% CI (0.08–0.93) p = 0.04) (Figure 1(c)).

Next, we examined the impact of ATB on the response rate. In the ATB group, none of the patients experienced a partial or complete response. The response rate defined by at least a partial response (PR) was 34% in the non-ATB group (p < 0.01) (Figure 2).

We performed a multivariate analysis of the effect of ATB administration taking into account standard prognostic factors relevant for advanced melanoma including age, ECOG status, gender, serum levels of lactate dehydrogenase (LDH), BRAF status, the line of therapy, and type of ICI (anti-PD1 vs anti-CTLA-4). The multivariate analysis further supported that ATB administration was associated with worse PFS (HR 0.32 (0.13–0.83) 95% CI, p = 0.02) (Table 2). Performance status also represented an independent risk factor for worsening PFS (HR 0.42 (0.24–0.77) 95% CI, p < 0.01). Performance status was the only predictive factor for OS in the multivariate analysis (HR 0.39 (0.20–0.76) 95% CI, p < 0.01) (Supplemental Table 2). Lastly, grade 3–4 immune-related toxicities occurred in 4.05% of the patients in the non-ATB group vs 1.35% in the ATB group (Supplemental Table 3). The incidence of severe immune-related toxicities was too small to report on a difference between the two arms.

**Discussion**

Our study found a non-negligible rate of ATB administration (over 13%) in this population of patients with advanced melanoma and also has demonstrated the potentially nefarious impacts on patient clinical outcomes. Indeed, in this cohort, ATB use within the 30 days prior to the initiation of ICI was associated with worse clinical outcomes. Our cohort had a relatively reduced incidence of ATB prescription in comparison to other studies assessing the impact of ATB use on ICI efficacy. However, it should be noted that the prescription of ATB may be more common in patients with lung cancer and renal cell cancer in comparison to those with melanoma, as we observed in other groups studying NSCLC where the rate of ATB prescription was as high as 36.7%.10

These findings support previous work demonstrating the negative prognostic impact of ATB in cancers treated with ICI. Two large retrospective studies of 249 and 239 NSCLC, RCC and UC treated with ICI demonstrated that ATB prescription was an independent predictor of worse outcome.8 A large retrospective study of 303 patients with advanced melanoma, NSCLC, and RCC treated with ICI further suggested the deleterious effect of ATB.10 In addition, Do et al. conducted a retrospective review of 109 patients with advanced lung cancer treated with nivolumab. The patients who received ATB had worse OS (5.4 months vs 17.2 months HR 0.29 p = 0.0004).14 Moreover, Huemer et al. demonstrated in their study of 30 patients that median OS was significantly shorter in the ATB group (7.5 vs 15.1 months HR 0.31 p = 0.026) with multivariate analysis confirming that ATB use was the only parameter of statistical significance associated with worse PFS and OS.15

These findings reinforce the hypothesis that ATB-induced dysbiosis of the gut microbiota is associated with a loss of commensal diversity, specifically with a decrease in Bacteroides isolates.16 This influence on the composition of the gut microbiota is determined by the class, duration, and route of ATB use. Furthermore, these perturbations were found to be potentially reversible after 16S ribosomal RNA sequencing demonstrated a restoration of gut microbiota composition to baseline within 1–3 months and in some rare cases after years.17–19

Wargo’s group from MD Anderson used 16S RNA sequencing technology on feces from 43 melanoma patients to demonstrate that Faecalibacterium spp. were overrepresented in responder patients.11 Further confirming that the gut microbiome composition influences clinical response in melanoma patients, Matson et al. showed that Bifidobacterium longum and adolescentis, Collinsella aerofaciens, and Parabacteroides merdae were more abundant in the stools from patients that responded to ICI as compared to patients that did not respond to ICI, in which Ruminococcus obeum and Roseburia intestinalis were more abundant.12

Exploring the effects of the gut microbiota composition on the toxicity of anti-CTLA-4 therapy in patients with metastatic melanoma, two separate groups demonstrated that baseline fecal samples with an abundance of Bacteroidetes phylum and an absence of Firmicutes was associated with a decreased incidence of ICI-induced auto-immune colitis.20,21 In
addition, modification of the gut microbiome through fecal microbial transplantation might represent a novel therapeutic avenue for steroid-refractory immune-related colitis.²²

Our study has several limitations, such as the retrospective nature of the data, small sample size resulting in some imbalance between the two groups, as well as the collection from two separate institutions. However, implementing the RECIST 1.1 criteria in both centers patients’ clinical responses allowed for standardization and objectivity. Moreover, the additional factors with a possible influence on the composition of the gut microbiota such as diet, country of origin, specific co-morbidities or concomitant medications were not included in the analyses. Furthermore, the mechanism by which ATB exert a detrimental effect was not delineated as we speculated that ATB-related dysbiosis decreases the taxonomic richness of the gut microbiota and eradication of the immunogenic bacteria required to invigorate the immune system during ICI treatments. In future cohorts, efforts to obtain and analyze samples from tumors and peripheral blood should be attempted. Lastly, whether ATB use reflects a general prognostic association or is causal to resistance to ICI remains a matter of debate.

Nevertheless, the multivariate analysis showing shorter PFS in groups with ATB use supports the independent association of ATB with worse clinical outcomes. In addition, ATB-based conditioning of tumor-bearing mice with the same genetic background blunts the efficacy of PD-1 or PD-1+CTLA-4 blockade in otherwise “normal” animals, suggesting a causality between ATB and primary resistance to ICI and immunogenic chemotherapy.²⁴ Lastly, in our study, the frequency of immune-related adverse effects was too small and we were unable to evaluate the association between ATB and adverse events.

In conclusion, these findings suggest that ATB use in patients with advanced melanoma is associated with adverse patient outcomes. These results support the hypothesis that ATB-induced dysbiosis may impair the ability of ICI to invigorate the immune system and can potentially cause ICI resistance. Additional studies are needed to delve further into the causal relationship between ATB use and ICI efficacy. However, based on our current understandings, ATB stewardship and judicious use of ATB should be implemented in patients with advanced melanoma receiving ICI. Prospective studies will be important in order to determine whether ICI initiation could safely be delayed beyond 30 days after the course of necessary ATB. In addition, the beneficial role of probiotics post-ATB remains controversial.¹⁵ Interventions to promote microbiota recovery post-ATB should also be studied in randomized clinical studies. This study raises the question of the potential need for microbiota profiling in cancer patients and the clinical relevance of the gut microbiome as a novel biomarker of ICI efficacy and immune-related toxicities.

**Methods**

**Patients**

A total of 74 patients with advanced melanoma were identified at the Centre de recherche du Centre hospitalier de l’Université de Montréal (CRCHUM) and at the Jewish General Hospital (JGH) in Montreal, Canada. Eligible patients were men and women.

**Table 1. Baseline characteristics of patients with advanced melanoma.**

| Characteristics               | Total (N = 74) | ATB (p = 10) | No ATB (n = 64) | p-Value |
|-------------------------------|----------------|--------------|-----------------|---------|
| Age-yr                        | 18–84          | 23–77        | 18–84           |         |
| Age-yr-no. (%)                |                |              |                 |         |
| <65                           | 37 (50.0)      | 6 (60.0)     | 31 (48.4)       | <0.75   |
| ≥65                           | 37 (50.0)      | 4 (40.0)     | 33 (51.6)       |         |
| Gender-no. (%)                |                |              |                 |         |
| Male                          | 40 (54.1)      | 6 (60.0)     | 34 (53.1)       | <0.75   |
| Female                        | 34 (45.9)      | 4 (40.0)     | 30 (46.9)       |         |
| ECOG performance status – no. (%) |          |              |                 |         |
| 0                             | 38 (51.4)      | 3 (30.0)     | 35 (54.7)       | <0.27   |
| 1                             | 33 (44.6)      | 6 (60.0)     | 27 (42.2)       |         |
| 2                             | 3 (4.0)        | 1 (10.0)     | 2 (3.1)         | <0.40   |
| Histology – no. (%)           |                |              |                 |         |
| BRAF+                         | 10 (13.5)      | 0            | 10 (15.6)       | <0.39   |
| Not reported                  | 8 (10.8)       | 1 (10.0)     | 6 (9.4)         |         |
| Prior lines of therapy – no. (%) |          |              |                 | <0.90   |
| 0                             | 49 (66.2)      | 7 (70.0)     | 42 (65.5)       |         |
| 1                             | 11 (14.9)      | 1 (10.0)     | 10 (15.6)       |         |
| ≥2                            | 14 (18.9)      | 2 (20.0)     | 12 (18.8)       |         |
| LDH – no. (%)                 |                |              |                 |         |
| ≤190                          | 47 (63.5)      | 8 (80.0)     | 38 (59.4)       | <0.24   |
| >190                          | 23 (31.1)      | 1 (10.0)     | 23 (35.9)       |         |
| Site of metastases – no. (%)  |                |              |                 | <0.27   |
| Lung                          | 36 (48.6)      | 3 (30.0)     | 33 (51.6)       |         |
| Liver                         | 23 (31.1)      | 3 (30.0)     | 20 (31.3)       |         |
| Brain                         | 6 (8.12)       | 1 (10.0)     | 5 (7.8)         |         |
| Other                         | 8 (11.22)      | 3 (30.0)     | 6 (9.4)         |         |
| Treatment-no.%                |                |              |                 | <0.13   |
| Pembrolizumab                 | 41 (55.4)      | 5 (50.0)     | 36 (56.3)       |         |
| Nivolumab                     | 13 (17.6)      | 0            | 13 (20.3)       |         |
| Ipilimumab                    | 5 (6.75)       | 2 (20.0)     | 3 (4.69)        |         |
| Combination ipilimumab + chemotherapy | 15 (20.2) | 3 (30.0) | 12 (18.8) | <0.85 |
| Treatment after immunotherapy |                |              |                 |         |
| No subsequent treatment       | 63 (85.1)      | 10 (100.0)   | 53 (82.8)       |         |
| Therapy targeting MAPK        | 2 (2.7)        | 0            | 2 (3.1)         |         |
| Chemotherapy                  | 4 (5.4)        | 0            | 4 (6.3)         |         |
| Local RT                      | 1 (1.4)        | 0            | 1 (1.6)         |         |
| Other immunotherapy           | 2 (2.7)        | 0            | 2 (3.1)         |         |
| Clinical trial                | 2 (2.7)        | 0            | 2 (3.1)         |         |
≥18 years of age with a histologic diagnosis of metastatic melanoma treated with anti-PD-1 mAb nivolumab or pembrolizumab, or an anti-CTLA-4 mAb alone as part of the standard of care treatment regimen or in combination with chemotherapy as part of the protocol NCT01676649. Prior treatment with BRAF inhibitors in the metastatic setting was permitted. Patient inclusion required the presence of measurable or evaluable disease. For patients studied as part of clinical trial NCT01676649, adequate bone marrow, liver, and renal function at study entry were additional inclusion criteria. Specific exclusion criteria for our study included an ECOG performance status of >2 and no prior line of immunotherapy. For the patients studied as part of clinical trial NCT01676649, additional exclusion criteria included: adequate bone marrow, liver, and renal function at study entry, autoimmune disease, peripheral neuropathy ≥ Grade 2, prior treatment with a CD137 agonist or a CTLA-4 agonist/inhibitor, and chronic use of immunosuppressive drugs or of systemic corticosteroids.

After appropriate ethics approval at both institutions, patient records were retrospectively analyzed to identify the use of oral or intravenous ATB within 30 days prior to the initiation of ICI. The class of ATB, indication, route of administration and duration were recorded. Clinicopathologic characteristics including age, gender, histology, Eastern

Figure 1. Progression-free survival (a), overall survival (b) patients with advanced melanoma treated with ICI and also including those who also received chemotherapy in combination with ICI (n = 74), stratified by use of ATB within 30 days of initiating ICI, overall survival (c) in patients who received ICI alone without chemotherapy (n = 59). The p-values calculated with chi-squared and log-rank tests.

Figure 2. Impact of ATB use on the response rate of therapy. CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease.

Table 2. Progression free-survival multivariate analysis.

| Variables                  | Hazard Ratio | CI 95%     | p-Value |
|----------------------------|--------------|------------|---------|
| ATB No vs Yes              | 0.32         | 0.13–0.83  | 0.02    |
| Age ≤65 vs >65             | 0.93         | 0.52–1.63  | 0.79    |
| ECOG 0 vs 1–2              | 0.42         | 0.24–0.77  | 0.01    |
| Gender M vs F              | 0.95         | 0.50–1.79  | 0.87    |
| LDH <190 vs. ≥190          | 0.95         | 0.54–1.66  | 0.84    |
| BRAF wildtype vs mutated   | 0.94         | 0.38–2.35  | 0.90    |
| Line of Tx 0 vs ≥1         | 1.60         | 0.77–3.34  | 0.21    |
| PD-1 vs Ipi                | 0.76         | 0.39–1.48  | 0.42    |
Cooperative Oncology Group (ECOG) performance status, prior lines of therapy, serum lactate dehydrogenase level (LDH) and treatment type were recorded for all patients.

For the evaluation of tumor response, CT scans were reviewed by local specialized radiologists and response was determined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. All patients were followed-up until death or data lock of May 1, 2018.

Immune-related toxicities were recorded according to the Common Terminology Criteria for Adverse Events (CTCAE) guideline as is used in clinical practice.

**Statistical analysis**

Patient characteristics were described according to ATB usage (ATB vs non-ATB). These data points were compared with the Fisher or Chi-squared tests for categorical data and t-test for continuous data. Progression-free survival (PFS) was defined as the time from the initiation of ICI to the first event (tumor progression or death from any cause) and overall survival (OS) was defined as the time from ICI initiation to death from any cause. Patients with no events were censored at the date of the last follow-up.

Differences in best overall response were analyzed using Chi-squared test. Survival curves were estimated by the Kaplan-Meier method and compared with the Log-rank test (univariate analysis). Univariate hazard ratios were calculated using the Log-rank method. Multivariable Cox regression model was used to determine hazard ratios and 95% confidence intervals for PFS and OS between ATB and non-ATB, adjusting for clinicopathologic features including: age, ECOG performance status, gender, LDH, BRAF, the line of treatment, and type of immunotherapy (anti-PD-1 vs anti-CTLA-4). Statistical tests were two-sided and a p-value less than 0.05 was considered statistically significant. Statistical analyses were carried out using SPSS.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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