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## **Baseline co-medications may alter the anti-tumoral effect of checkpoint inhibitors as well as the risk of immune-related adverse events**

**Running head:** Baseline co-medications may alter efficacy of ICI

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## **ABSTRACT**

### **PURPOSE**

Since gut microbiota composition is an important determinant of response to immune checkpoint inhibitors (ICI), we examined the effect of various co-medications known for their interaction with microbiota, when given at ICI initiation.

### **PATIENTS AND METHODS**

We identified patients with advanced cancer treated with ICI between May 2015 and September 2017 in our institution. Co-medications given within one month before or one month after the first administration of ICI were reviewed from medical records. Survival data were analyzed with univariable Cox regression and the combined effect of multiple factors was assessed with factor analysis of mixed data (FAMD). The impact of co-medications on immune-related adverse events (irAEs) occurrence was also assessed.

### **RESULTS**

635 patients were included. Psychotropic drugs (41%), proton pump inhibitors (PPIs) (38%), angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockers (ARBs) (32%), glucocorticoids (26%), antibiotics (24%), statins (21%) and morphine (20%) were the most prescribed co-medications. Baseline use of antibiotics, glucocorticoids >10mg/day, PPIs, psychotropic drugs, morphine and insulin was associated with significantly shortened overall survival and decreased tumor response, while coadministration of statins, ACEs and/or ARBs, NSAID, aspirin and oral anti-diabetic drugs did not impact patients outcomes. Treatments that altered the response to ICI were also

associated with a decreased incidence of irAEs. FAMD revealed the respective weight of each factor or comedication on the oncological outcomes.

## **CONCLUSION**

Co-medications must be carefully assessed at the time of ICI initiation and clinicians aware of their possible deleterious effect, notably for PPIs, glucocorticoids, antibiotics and psychotropic drugs.

## **INTRODUCTION**

Immune checkpoint inhibitors (ICI) are revolutionizing the treatment of advanced cancers (1,2). Several studies have demonstrated that the gut microbiota composition may be an important factor explaining why not all patients respond adequately to ICI (3–5). Moreover, it has been shown that microbiota composition can also be related to the occurrence of immune related adverse events frequently observed under ICI (6,7). Progression-free survival (PFS) and overall survival (OS) were significantly shorter in lung, kidney and bladder cancer patients who received antibiotics for routine reasons one or two months before or soon after starting an anti PD-1 drug, suggesting that simply avoiding antibiotics while starting PD-1 inhibitors could boost patient responses from 25% to 40% (5). Then, such observation has been reproduced by several groups (8) and led to the concept that manipulations of the gut microbiota could serve to enhance the efficacy of immune checkpoint inhibitors in cancer treatment (9,10). If antibiotic treatments obviously alter the gut microbiota, strongly modifying its composition, reducing its microbial diversity and promoting colonization by pathogens, non-antibiotic drugs may also have an important impact on this microbiota (11,12). In this way, anti-diabetics, proton pump inhibitors (PPIs), aspirin, nonsteroidal anti-inflammatory drugs (NSAIDS), steroids, immunomodulators, psychotropic drugs and analgesics such as opioids have been associated with significant changes in microbiome composition (13,14). Thus, as many patients suffering from advanced cancer are exposed to such drugs, our aim was to investigate the possible influence of these co-medications on the anti-tumor effect and safety of ICI.

## **METHODS**

### ***Study design***

This was a retrospective cohort study conducted in Bordeaux University Hospital, including patients treated with ICI.

### ***Patients***

All adult patients who received ICI from May 2015 to September 2017 were included in the study.

### ***Study objectives***

The primary objective was to estimate the impact of co-medications on OS after ICI onset. Secondary objectives were to evaluate their impact on 1) PFS, 2) best tumor response, collected and defined by the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria as read by a radiologist on serial CT imaging (15), and 3) immune-related adverse events (irAEs) occurrence.

### ***Data extraction***

Data were extracted from the medical file records of each patient included.

Co-medications given to the patient within one month before and after the first administration of ICI were assessed according to a predefined list of medications known to impact gut microbiota: antibiotics (including class, duration, route of administration, therapeutic or prophylactic use and hospitalization), glucocorticoids (GCs) (including dose, route of administration and indication), metformin and other oral antidiabetic drugs, insulin, PPIs, aspirin, NSAIDs, statins, angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARBs), immunomodulators, psychotropic drugs, hormone replacement therapies and morphine.

Known prognostic factors for melanoma (ECOG performance status, BRAF status, LDH and stage  $\geq$  M1c), lung cancer (ECOG performance status, brain metastasis, EGFR, smoking status, histology) and for renal cell carcinoma (IMDC risk score) were collected.

OS and PFS were assessed from the start of ICI until September 2019 or last follow-up.

### ***Statistical analysis***

Qualitative data were analyzed between groups using Chi-2 test or Fisher's exact test. Patients were considered as responders when complete response, partial response or stable disease was obtained, and as non-responders in case of progressive disease. Survival data were analyzed with univariable Cox regression and presented using Kaplan-Meier's graphics. When patients received sequential ICI therapies, we considered the first ICI. Analyses were computed on STATA 13.1 software (StataCorp LP, College Station, TX) with a statistically significant threshold of 0.05. Graphs were constructed using GraphPad Prism software version 6.

Considering the large number of factors susceptible to influence the treatment response, we performed a factor analysis of mixed data (FAMD), which is a principal component method balancing the influence of all the quantitative and qualitative variables in the construction phase of the dimensions of variability. The aim of using this method is to find similarities between individuals, the relationships between variables and to link the study of the individuals with that of the variables. This method reduces the dimensionality of the data and provides the subspace that best represents the dataset. According to their response to ICIs, we first categorized patients as poor, intermediate or long survival patients. Then, we considered separately the main types of cancer (melanoma, lung and renal), in order to include their respective prognostic biomarkers. Imputations methods for missing data were



not used since they are based on mean values while our dataset contained mainly categorical variables. All samples containing at least one missing value have been discarded. This FAMD was performed by using the R package FactoMineR (version 2.4).

## RESULTS

### *Patients*

635 patients were included. Baseline characteristics and prevalence of co-mediations are shown in **Table 1**. Only co-mediations with prevalence  $\geq 3\%$  were then analyzed. Patient characteristics according to co-mediations of interest are described in **Table S1**. Overall, median OS was 16 months, median PFS was 5 months, and 399/635 (63%) patients died. Tumor response occurred in 387/569 patients (68%), including complete response (n=37; 7%), partial response (n=156; 27%) or stable disease (n=194; 34%), while progressive disease was observed in 182 patients (32%).

### *Impact of co-mediations on survival and tumor response*

#### **Antibiotics**

At the time of CPI initiation, 150 patients (24%) were given antibiotics, oral (n=97), intravenous (n=42) or topical (n=3). For 22 patients (15%), antibiotics were prescribed for prophylactic use. The most prescribed class was beta-lactam (n=103, 69%), followed by sulfonamide (n=25, 17%), quinolone (n=20, 13%), macrolide (n=18, 12%), aminoglycoside (n=11, 7%) and tetracycline (n=8, 5%). The majority of patients received one class of antibiotics (n=96, 64%), 41 patients (27%) receiving two and 13 patients (9%)  $\geq 3$  classes of antibiotics. Hospitalization was required for 58 patients (40%). Median OS was shorter for patients receiving antibiotics compared to patients who did not (8 versus 23 months;

$p < 0.001$ ; HR, 1.66; 95%CI, 1.34-2.07) (**Figure 1A and Table 2**). Similarly, median PFS was negatively impacted in patients receiving antibiotics (4 versus 7 months,  $p = 0.04$ ; HR, 1.27; 95%CI, 1.01-1.59) (**Table S2**). Tumor response occurred in 57% of patients who received antibiotics compared to 71% of patients without antibiotics ( $p = 0.004$ ) (**Figure 2**).

### **Glucocorticoids (GCs)**

When starting ICI treatment, 195 patients (31%) were receiving GCs, 165 patients (26%) had systemic GCs and 30 patients (5%) topical GCs. Systemic GCs were prescribed for brain metastases ( $n = 61$ , 39%), other metastases ( $n = 35$ , 22%), associated chemotherapy ( $n = 16$ , 10%), pain ( $n = 12$ , 8%), poor performance status ( $n = 6$ , 4%), or various non-oncological indications ( $n = 28$ , 18%). Baseline systemic GCs use was associated with significant decreased of both OS (median 5 versus 26 months;  $p < 0.001$ ; HR, 1.46; 95%CI, 1.26-1.69) and PFS (median 3 versus 7 months;  $p = 0.005$ ; HR, 1.24; 95%CI, 1.07-1.44) (**Figure 1B, Table 2**). In order to assess whether different steroids dosages may differently impact patient outcomes, we stratified patients into 4 groups: no GC,  $\leq 10$ mg/day,  $> 10$ mg/day but  $\leq 20$ mg/day and  $> 20$ mg/day of prednisone equivalent. While low GCs dosage  $\leq 10$ mg/day did not significantly impact OS ( $p = 0.5$ ; HR, 1.12; 95%CI, 0.79-1.59), higher dosages were associated with poorer outcome ( $p < 0.001$ ; HR, 2.47; 95%CI, 1.57-3.88 between 10 and 20mg/day and  $p < 0.001$ ; HR, 2.97; 95%CI, 2.33-3.78 when  $> 20$ mg/day) (**Figure 3A and Table 2**). Similarly, PFS was significantly impacted with baseline dosage  $> 10$ mg/day (**Table S2**). Tumor response was less frequently observed for patients receiving GC (50% versus 79%,  $p = 0.0001$ ) (**Figure 2**).

Of note, 30 patients (4.7%) had inhaled or topical steroids, and we did not observe significant difference neither in OS nor PFS compared to patients without GC (median OS 18 versus 26 months;  $p=0.6$  and 6.5 versus 7 months;  $p=0.35$  respectively) (**Figure S1**).

Then, we aimed to evaluate whether the use of GCs given not at ICI onset but later for irAE management may also impact survival. While median OS of patients treated with GCs at the start of ICI was 5 months, it was 48 months in patients treated with GCs later for irAE management ( $p<0.0001$ )(**Figure 3B**). Median PFS of patients with baseline GCs was 3 months but 9 months if GCs were used later for irAE management ( $p=0.004$ ).

### **PPIs**

239 patients (38%) were receiving PPIs. Most prescribed PPIs were esomeprazole ( $n=141$ , 59%), at 20mg/day (60%) or higher dose (40%), and omeprazole ( $n=58$ , 24%) at 20mg/day (82%) or higher dosage (18%). Pantoprazole was prescribed in 23 patients at 20mg/day (40%) or 40mg/day (60%), lansoprazole in 8 patients and rabeprazole in 7 patients. Comparing patients receiving PPIs or not, median OS was 9 versus 26.5 months ( $p<0.001$ ; HR, 1.70; 95%CI, 1.40-2.08) (**Figure 1C and Table 2**). PFS was also significantly reduced for patients with baseline PPIs (3.5 versus 8 months;  $p=0.002$ ; HR 1.37; 95%CI, 1.12-1.66) (**Table S2**). Tumor response was less frequently observed in patients receiving such co-medication (61% versus 72%,  $p=0.007$ ) (**Figure 2**).

### **Statins**

Statin was prescribed to 133 patients (21%). OS, PFS and tumor response were similar between patients receiving statin or not (**Figure 1D and 2, Table 2 and S2**).

### **Morphine**

130 patients (20%) were given morphine. Significant shorter median OS and PFS were observed in this group of patients compared to patients not treated with morphine (respectively 4 versus 25.5 months,  $p<0.001$ ; HR, 2.69; 95%CI, 2.15-3.38 and 3 versus 6.5 months,  $p<0.001$ ; HR, 1.58; 95%CI, 1.23-2.03) (**Figure S2A, Table 2 and S2**). Morphine treatment was also associated with a decreased rate of tumor response (51% versus 71%,  $p=0.0001$ ) (**Figure 2**).

### **NSAIDs**

63 patients (10%) received oral NSAIDs. Median OS was 10 months for these patients versus 20 months for patients not receiving NSAIDs ( $p=0.05$ ; HR, 1.36; 95%CI, 0.99-1.85) (**Figure S2B and Table 2**). Median PFS was similar in both groups (6 months,  $p=0.4$ ; HR, 1.15; 95%CI, 0.84-1.59) (**Table S2**). Tumor response rate was similar in both groups (72% versus 68%,  $p=0.5$ ) (**Figure 2**).

### **Aspirin**

109 (17%) patients were prescribed aspirin, with a median OS of 19.5 months versus 22 months ( $p=0.005$ ; HR, 1.42; 95%CI, 1.11-1.82), and a median PFS of 7 months versus 6 months for patients without aspirin ( $p=0.8$ ; HR 1.03; 95%CI, 0.80-1.33). Tumor response rate was similar in both groups (67% versus 68%,  $p=0.8$ ) (**Figure 1E and 2, Table 2 and S2**).

### **ACE inhibitors / ARBs**

OS, PFS and tumor response were not altered with baseline co-medication with ACE inhibitors and/or ARBs (**Figure 1F and 2, Table 2 and S2**).

### **Psychotropic drugs**

An important proportion of patients (41%) were treated with baseline psychotropic drugs, which significantly impacted OS (median 9 versus 28 months;  $p<0.001$ ; HR, 1.82; 95%CI, 1.49-2.22), PFS (median 4 versus 7 months;  $p=0.002$ ; HR, 1.36; 95%CI, 1.12-1.64) and tumor response (60% versus 73%,  $p=0.002$ ) (**Figure 1G and 2, Table 2 and S2**).

### **Metformin, other oral antidiabetic drugs and insulin**

Both OS and PFS were not impacted with baseline metformin regimen (median 20 months;  $p=0.9$ ; HR, 0.98; 95%CI, 0.64-1.50 and median 5 versus 6 months;  $p=0.9$ ; HR, 0.9; 95%CI, 0.64-1.44 respectively) (**Figure 1H, Table 2 and S2**) and tumor response rate was 74% versus 68% ( $p=0.4$ ) (**Figure 2**). Similar results were found with other oral antidiabetic drugs (**Figure S2C and 2, Table 2 and S2**).

Baseline co-medication with insulin ( $n=23$ ) associated with significant decreased in OS (median 6.5 versus 20 months;  $p=0.043$ ; HR, 1.68; 95%CI, 1.01-2.77) (**Figure S2D and Table 2**) but not in PFS (median 3.5 versus 6 months;  $p=0.8$ ; HR, 1.08; 95%CI, 0.63-1.84) (**Table S2**) nor with tumor response (67% versus 68%;  $p=0.9$ ) (**Figure 2**).

### ***Combination of treatments with significant impact on survival***

Prescription of PPIs, GCs and antibiotics that were shown to impact patient outcomes are frequently combined. Therefore, we assessed the impact of each medication alone and in combination (**Figure 3C**). Median OS was 4.5 months for patients receiving combination of PPIs and GCs versus 9 months when GCs were given alone ( $p=0.1$ ), 17 months when PPIs alone ( $p<0.0001$ ) and 32 months when none of these treatment were used ( $p<0.0001$ ).

Median PFS was 3 months for the combination PPIs and GCs, similar to GCs alone ( $p=0.8$ ), versus 5 months with PPIs alone ( $p=0.2$ ) and 8.5 months when none of these co-medications were given ( $p<0.0001$ ).

Median OS of patients receiving the combination of PPIs and antibiotics was 5.5 months versus 12.5 months when antibiotics were given alone ( $p=0.3$ ), 11 months when PPIs alone ( $p=0.1$ ) and 32 months with none of them ( $p<0.0001$ ). Same results were observed for PFS with a median of 3.25 months in the group combination PPIs and antibiotics, versus 4 months if antibiotics alone ( $p=0.9$ ), 3.5 months if PPIs alone ( $p=0.9$ ) and 8,5 months if none of these co-medications ( $p=0.02$ ).

#### ***Impact of co-medications on irAE occurrence***

Overall, 276/632 patients (43.7%) experienced irAEs, either one irAE ( $n=163$ ), two irAEs ( $n=79$ ) or  $\geq 3$  irAEs ( $n=34$ ), with a median exposure time of 52 days [30-91] for the first irAE. Most common irAEs were cutaneous ( $n=160$ , 25%), digestive/hepatic ( $n=79$ , 12.5%), endocrine ( $n=66$ , 10%) and rheumatic ( $n=49$ , 8%), followed by pulmonary ( $n=21$ , 3%), neurological ( $n=13$ , 2%), ocular ( $n=10$ , 1.5%), renal ( $n=7$ , 1%), hematological ( $n=1$ ) and cardiac ( $n=1$ ) irAEs. Notably, colitis occurred in 38 patients (6%).

Baseline co-medication with antibiotics, GCs, PPIs, morphine, NSAIDs, aspirin and psychotropic drugs was associated with decreased occurrence of irAE (**Table 3**). Notably, irAE occurred in 50% of patients (197/393) without PPIs while only in 33% of patients (79/239) receiving PPIs ( $p<0.001$ ). However, there was no difference in colitis occurrence according to PPIs treatment (6% in both groups). All but NSAIDs and aspirin were also associated with altered overall survival and tumor response. While insulin negatively impacted overall survival, it was not associated with change in irAE occurrence.

### ***Influence of all variables through a principal component analysis***

Factor analysis of mixed data was applied separately for melanoma, lung cancer and renal carcinoma patients, including demographics, prognostic biomarkers, co-medications, oncological outcomes and irAEs occurrence (**Figure 4 and Supplementary FAMD data**). Variable that contributed the most to the first dimension (x-axis) was survival, in all cancer types. Age was the main contributor to the second dimension (y-axis) for melanoma patients, as well as for lung cancer patients together with GCs, and it was tumor progression and IMDC for renal carcinoma patients. Position and colour of each factor on the plot represented its contribution value. Globally, compared to known prognostic factors, baseline use of GCs, antibiotics, PPIs and psychotropic drugs contributed to worse oncological outcome while irAE occurrence and GCs use for irAE contributed to better outcome, independently in all cancer types.

## **DISCUSSION**

First, our results confirm the detrimental impact on oncological outcomes of antibiotics and GCs at a dosage  $\geq 10$  mg/day when given within one month before or after ICI onset (5,16–18). Moreover, we show that other co-medications may significantly alter the antitumoral response of ICI, such as PPIs, psychotropic drugs, morphine, aspirin and insulin, while others seem to have no impact, such as statins, ACEs and/or ARBs, NSAIDs, and oral anti-diabetic drugs.

These results suggest some practical advice in a patient candidate to ICIs. First, antibiotic treatment should be limited to documented infections. Regarding baseline glucocorticoids

use, the cut-off of 10mg/day should be respected, considering the deleterious effect of higher dosage. Moreover, due to the lack of impact of inhaled or topical GCs, local routes should be preferred. Conversely, our study brings reassuring data regarding the use of GCs for the management of irAE(s), which did not alter ICI efficacy, confirming previous reports (19). Another message concerns the use of PPIs, considering that they are most often given for prevention, gastric discomfort and clearly over-prescribed. Such prescription should therefore be (re)-evaluated properly at the time of ICI initiation, in order to optimize patient outcomes. Between our data collection and publication of the manuscript, other groups have reported similar findings on PPIs, even with the development of drug-based prognostic score including exposure to GC, antibiotics and PPIs (20,21). Our data on drugs combination is in accordance with such score, revealing the cumulative negative effect of multiple drugs exposure. Of note, the negative impact of concomitant administration of PPIs has been also outlined with other drugs than ICI such as pazopanib in soft-tissue sarcoma patients (22).

On another hand, we found that some co-medications such as statins or ACE inhibitors/ARBs, clearly not impacted anti-tumoral efficacy of ICI, in accordance to Cortellini *et al* (20), while statins have been associated with improved clinical activity of ICI (23,24) and ACE inhibitors with impaired outcomes (25). Psychotropic drugs are an heterogeneous group of medications that should be evaluated separately. Synergistic effect of metformin in combination with ICI has been previously suggested but not observed neither in our study nor in those of Omori *et al* (24,26). Insulin strongly associated with poorer outcomes but on a small number of patients and further studies with particular interest in antidiabetic agents should be conducted before any recommendation.



The observational nature of our study does not allow any causal conclusion. Since our first aim was to evaluate the impact of co-medications known to modify gut microbiota, we do not know whether their effect on cancer outcomes is thoroughly mediated by changes in microbiota or other immunomodulatory properties. Microbiota is now recognized as a major intrinsic regulator of immune response and its composition influences response to ICIs in both humans and mice, mainly through the induction of IFN $\gamma$ + CD8 T cells and NKT cells (27–29). This pharmacometabolomic concept holds the immense potential of gut manipulation to improve ICI efficacy. The first in-human studies of fecal microbiota transplant (FMT) before anti PD-1 rechallenge in refractory melanoma patients were recently published, inducing rapid changes in gut microbiota with clinical response in some patients (30,31). They represent a proof-of-concept that manipulation of gut microbiota may overcome resistance to anti PD-1 treatment, and other clinical trials are ongoing (NCT04130763, NCT04729322, NCT03341143). Probiotic therapy and high-fiber, plant-based diet are also studied, as more acceptable ways to induce favorable changes in gut microbiota to enhance ICI efficacy (32,33). Finally, introducing or withdrawing co-medications according to their positive or negative impact on ICI's response may represent another feasible option in clinical practice, therefore subject of active research highlighted by the growing number of publications during the last two years. The positive influence of some co-medications has been highlighted, as shown for dual antiplatelet therapy by aspirin and clopidogrel enhancing responsiveness to PD-1 blockade (34). Since only few patients were receiving clopidogrel in our cohort, we could not perform such analyses.

All the treatments that altered the anti-tumoral response to ICI were interestingly also associated with a decreased incidence of irAEs. Since there is a growing literature on the relationship between irAE occurrence and tumor response (35–39), our data are in

accordance with such idea and suggest that microbiota may also play a role in irAE development (40).

This study acknowledges several limitations beyond its retrospective and monocentric design, with reporting bias and missing data on baseline comedications, specific prognostic factors and cancer outcomes. We noticed differences between cancer types, with a negative impact of several co-medications only observed in melanoma patients highly represented in our cohort. Therefore focusing on one cancer type with a multicentric design or unplanned pooled post hoc analyses of clinical trials are of interest (41,42). One may also consider that currently ICI are more often prescribed as first-line treatment, notably in lung cancer patients (only 9 out of 150 patients in our cohort), and that previous systemic treatments may represent an additional bias in the interpretation of our results.

Finally, considering that the identification of prognostic factors of ICI response and improving its efficacy remains a major challenge, baseline co-medications should be carefully assessed at the time of ICI initiation and clinicians aware of their possible deleterious effect.

#### **References:**

1. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012 Mar 22;12(4):252–64.
2. Ribas A, Tumeo PC. Cancer therapy: Tumours switch to resist. *Nature*. 2012 Oct 18;490(7420):347–8.
3. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. 2015 Nov 27;350(6264):1079–84.
4. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*. 2015 Nov 27;350(6264):1084–9.

5. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. 2018 Jan 5;359(6371):91–7.
6. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol*. 2017 Jun 1;28(6):1368–79.
7. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med*. 2018 Dec;24(12):1804–8.
8. Wilson BE, Routy B, Nagrial A, Chin VT. The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. *Cancer Immunol Immunother*. 2020 Mar;69(3):343–54.
9. Pitt JM, Vétizou M, Waldschmitt N, Kroemer G, Chamillard M, Boneca IG, et al. Fine-Tuning Cancer Immunotherapy: Optimizing the Gut Microbiome. *Cancer Res*. 2016 15;76(16):4602–7.
10. Jobin C. Precision medicine using microbiota. *Science*. 2018 05;359(6371):32–4.
11. Keeney KM, Yurist-Doutsch S, Arrieta M-C, Finlay BB. Effects of antibiotics on human microbiota and subsequent disease. *Annu Rev Microbiol*. 2014;68:217–35.
12. Walsh J, Griffin BT, Clarke G, Hyland NP. Drug-gut microbiota interactions: implications for neuropharmacology. *Br J Pharmacol*. 2018 Dec;175(24):4415–29.
13. Maier L, Typas A. Systematically investigating the impact of medication on the gut microbiome. *Curr Opin Microbiol*. 2017 Oct;39:128–35.
14. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. *Gut*. 2016 May;65(5):740–8.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009 Jan;45(2):228–47.
16. Elkrif A, El Raichani L, Richard C, Messaoudene M, Belkaid W, Malo J, et al. Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors. *Oncoimmunology*. 2019;8(4):e1568812.
17. Arbour KC, Mezquita L, Long N, Rizvi H, Auclin E, Ni A, et al. Impact of Baseline Steroids on Efficacy of Programmed Cell Death-1 and Programmed Death-Ligand 1 Blockade in Patients With Non-Small-Cell Lung Cancer. *J Clin Oncol*. 2018 Oct 1;36(28):2872–8.
18. Scott SC, Pennell NA. Brief report: Early use of systemic corticosteroids in patients with advanced NSCLC treated with nivolumab. *J Thorac Oncol*. 2018 Jun 20;

19. Garant A, Guilbault C, Ekmekjian T, Greenwald Z, Murgoi P, Vuong T. Concomitant use of corticosteroids and immune checkpoint inhibitors in patients with hematologic or solid neoplasms: A systematic review. *Crit Rev Oncol Hematol*. 2017 Dec;120:86–92.
20. Cortellini A, Tucci M, Adamo V, Stucci LS, Russo A, Tanda ET, et al. Integrated analysis of concomitant medications and oncological outcomes from PD-1/PD-L1 checkpoint inhibitors in clinical practice. *J Immunother Cancer*. 2020 Nov;8(2).
21. Buti S, Bersanelli M, Perrone F, Tiseo M, Tucci M, Adamo V, et al. Effect of concomitant medications with immune-modulatory properties on the outcomes of patients with advanced cancer treated with immune checkpoint inhibitors: development and validation of a novel prognostic index. *Eur J Cancer*. 2020 Nov 16;142:18–28.
22. Mir O, Touati N, Lia M, Litière S, Le Cesne A, Sleijfer S, et al. Impact of Concomitant Administration of Gastric Acid-Suppressive Agents and Pazopanib on Outcomes in Soft-Tissue Sarcoma Patients Treated within the EORTC 62043/62072 Trials. *Clin Cancer Res*. 2019 Feb 14;
23. Cantini L, Pecci F, Hurkmans DP, Belderbos RA, Lanese A, Copparoni C, et al. High-intensity statins are associated with improved clinical activity of PD-1 inhibitors in malignant pleural mesothelioma and advanced non-small cell lung cancer patients. *Eur J Cancer*. 2021 Feb;144:41–8.
24. Omori M, Okuma Y, Hakozaiki T, Hosomi Y. Statins improve survival in patients previously treated with nivolumab for advanced non-small cell lung cancer: An observational study. *Mol Clin Oncol*. 2019 Jan;10(1):137–43.
25. Medjebar S, Truntzer C, Perrichet A, Limagne E, Fumet J-D, Richard C, et al. Angiotensin-converting enzyme (ACE) inhibitor prescription affects non-small-cell lung cancer (NSCLC) patients response to PD-1/PD-L1 immune checkpoint blockers. *Oncoimmunology*. 2020 Oct 27;9(1):1836766.
26. Afzal MZ, Mercado RR, Shirai K. Efficacy of metformin in combination with immune checkpoint inhibitors (anti-PD-1/anti-CTLA-4) in metastatic malignant melanoma. *J Immunother Cancer*. 2018 Jul 2;6(1):64.
27. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014 Mar 27;157(1):121–41.
28. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science*. 2018 25;360(6391).
29. Tanoue T, Morita S, Plichta DR, Skelly AN, Suda W, Sugiura Y, et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature*. 2019;565(7741):600–5.
30. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*. 2021 Feb 5;371(6529):602–9.

31. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin J-M, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*. 2021 Feb 5;371(6529):595–602.
32. Si W, Liang H, Bugno J, Xu Q, Ding X, Yang K, et al. *Lactobacillus rhamnosus* GG induces cGAS/STING- dependent type I interferon and improves response to immune checkpoint blockade. *Gut*. 2021 Mar 8;
33. Lee KA, Shaw HM, Bataille V, Nathan P, Spector TD. Role of the gut microbiome for cancer patients receiving immunotherapy: Dietary and treatment implications. *Eur J Cancer*. 2020 Oct;138:149–55.
34. Riesenber BP, Ansa-Addo EA, Gutierrez J, Timmers CD, Liu B, Li Z. Cutting Edge: Targeting Thrombocytes to Rewire Anticancer Immunity in the Tumor Microenvironment and Potentiate Efficacy of PD-1 Blockade. *J Immunol*. 2019 Sep 1;203(5):1105–10.
35. Toi Y, Sugawara S, Kawashima Y, Aiba T, Kawana S, Saito R, et al. Association of Immune-Related Adverse Events with Clinical Benefit in Patients with Advanced Non-Small-Cell Lung Cancer Treated with Nivolumab. *Oncologist*. 2018 Jun 22;
36. Bertrand A, Kostine M, Barnetche T, Truchetet M-E, Schaefferbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. *BMC Med*. 2015 Sep 4;13:211.
37. Haratani K, Hayashi H, Chiba Y, Kudo K, Yonesaka K, Kato R, et al. Association of Immune-Related Adverse Events With Nivolumab Efficacy in Non-Small-Cell Lung Cancer. *JAMA Oncol*. 2018 Mar 1;4(3):374–8.
38. Sato K, Akamatsu H, Murakami E, Sasaki S, Kanai K, Hayata A, et al. Correlation between immune-related adverse events and efficacy in non-small cell lung cancer treated with nivolumab. *Lung Cancer*. 2018;115:71–4.
39. Judd J, Zibelman M, Handorf E, O’Neill J, Ramamurthy C, Bentota S, et al. Immune-Related Adverse Events as a Biomarker in Non-Melanoma Patients Treated with Programmed Cell Death 1 Inhibitors. *Oncologist*. 2017;22(10):1232–7.
40. Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen W-S, Derosa L, et al. Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. *Nat Med*. 2021 Jul 8;
41. Cortellini A, Di Maio M, Nigro O, Leonetti A, Cortinovis DL, Aerts JG, et al. Differential influence of antibiotic therapy and other medications on oncological outcomes of patients with non-small cell lung cancer treated with first-line pembrolizumab versus cytotoxic chemotherapy. *J Immunother Cancer*. 2021 Apr;9(4).
42. Chalabi M, Cardona A, Nagarkar DR, Dhawahir Scala A, Gandara DR, Rittmeyer A, et al. Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled post hoc analyses of the OAK and POPLAR trials. *Ann Oncol*. 2020 Apr;31(4):525–31.

## Figure Legends

**Figure 1.** Overall survival of patients treated with checkpoint inhibitors on the basis of baseline coadministration of antibiotics **(A)**, glucocorticoids **(B)**, proton pump inhibitors **(C)**, statins **(D)**, aspirin **(E)** angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARBs) **(F)**, psychotropic drugs **(E)** and metformin **(F)**.

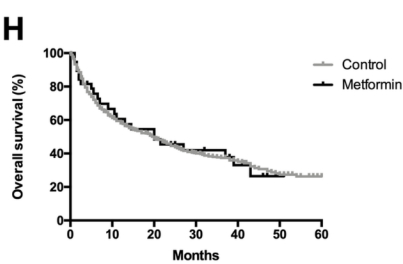
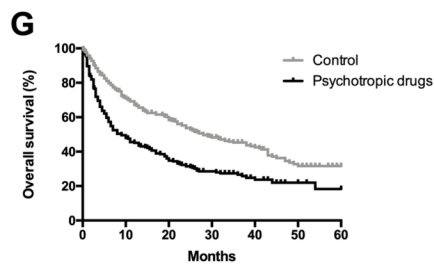
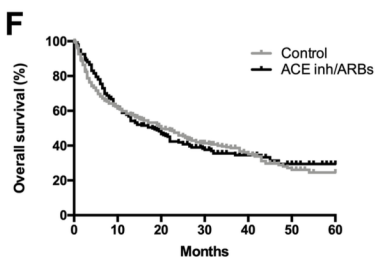
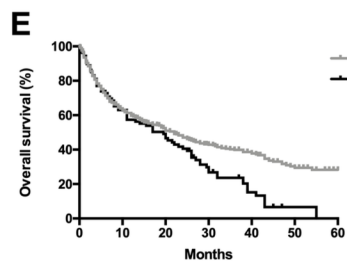
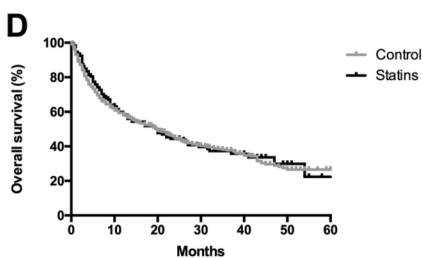
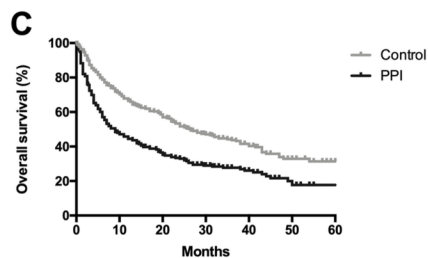
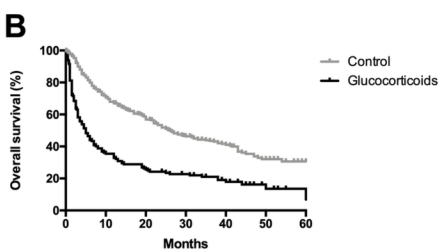
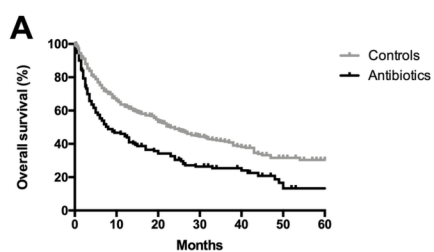
**Figure 2.** Overall response rate defined by the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria according to baseline co-medications. ACEinh/ARBs: angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers; AD: anti diabetic drugs; GCs: glucocorticoids; NSAIDs: non-steroidal anti-inflammatory drugs; PPIs: proton pump inhibitors.

**Figure 3.** Overall survival of patients treated with checkpoint inhibitors according to baseline glucocorticoids dosage **(A)**, use of baseline glucocorticoids or for immune-related adverse-events **(B)**, use of glucocorticoids in combination with proton pump inhibitors compared to both medications alone **(C)**.

**Figure 4.** Squared loading plot with visualization of categorical variables in melanoma patients and their role accounting for overall variation of this group of patients. Survival contributed the most to the dimension 1 (x-axis) and age was the main contributor to the dimension 2 (y-axis). Position and colour of each variable on the plot represented its contribution value and its relationship with other variables. 60: 60 years old; ACEinh/ARBs: angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers; GCs: glucocorticoids; ICI: immune checkpoint inhibitors; irAEs: immune-related adverse events followed by the number of irAEs; M1c: stage IV M1c; NSAIDs: non-steroidal anti-

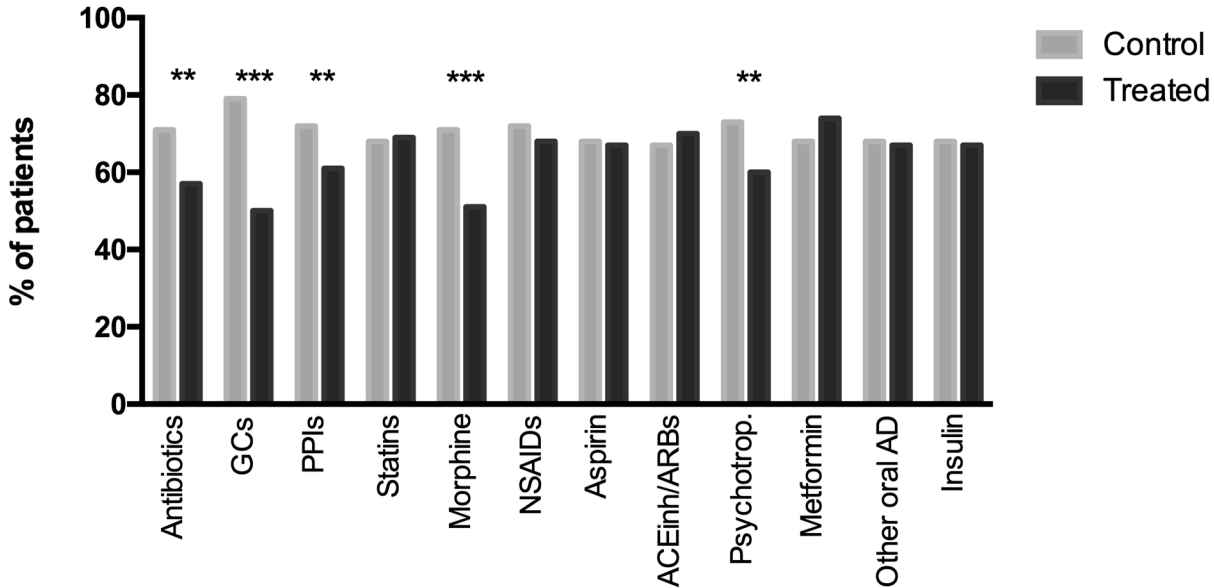
inflammatory drugs; PAD: pre-existing autoimmune disease; PFS: progression-free survival;

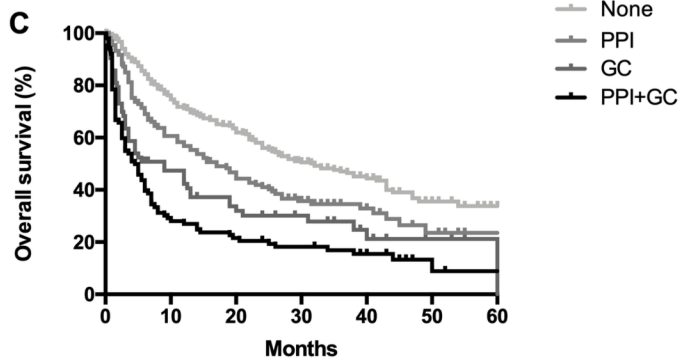
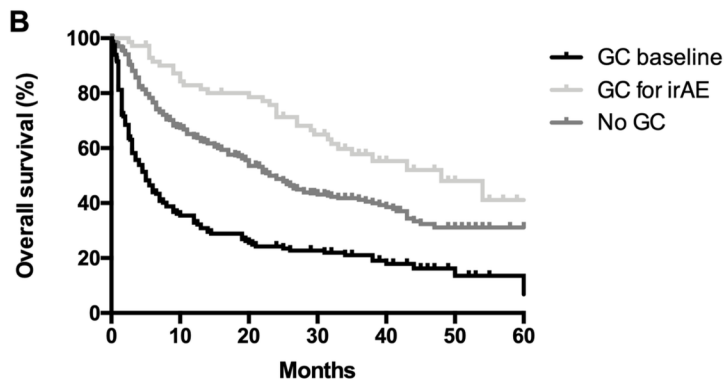
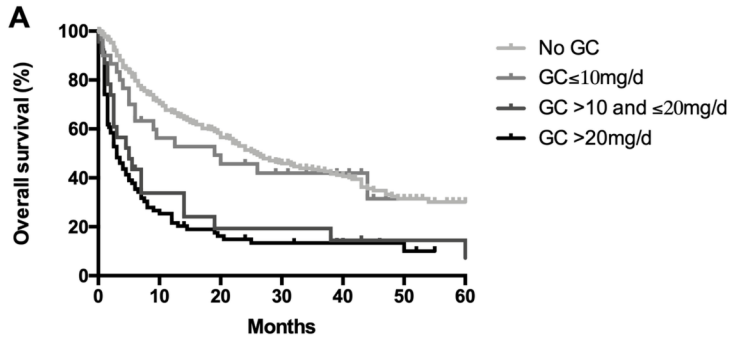
PPIs: proton pump inhibitors; PS: performance status.



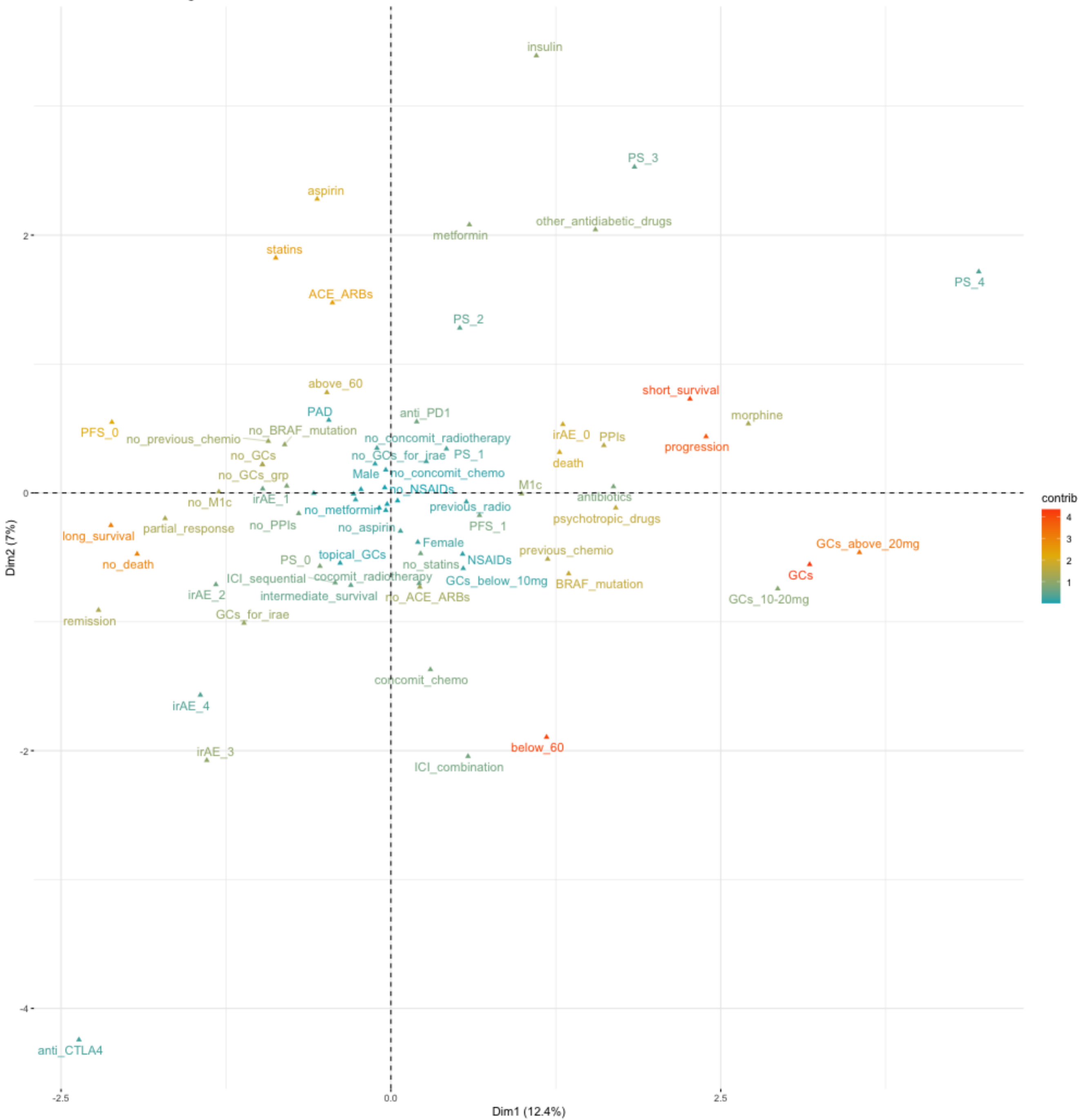


# Overall Response Rate





Qualitative variable categories - FAMD



**Table 1. Baseline characteristics of patients**

	n (%)
Age, mean (SD)	64.5 (13.1)
Gender	
Male	443 (70)
Female	192 (30)
Previous autoimmune disorders	
No	584 (92)
Yes	51 (8)
ECOG performance status	
0-1	528 (84)
≥ 2	98 (16)
Cancer type	
Melanoma	293 (46)
NSCLC	150 (24)
Renal	83 (13)
Head and neck	48 (8)
Blood cancer	20 (3)
Urothelial	16 (2.5)
Gastrointestinal/liver	16 (2.5)
Others	9 (1)
CPI therapy	
Anti CTLA-4	3 (0.5)
Anti PD-1	435 (68.5)
Anti PD-L1	66 (10)
Sequential CPI	100 (16)
Combined	31 (5)
Previous cancer therapy	
Chemotherapy/targeted therapy	
No	213 (33.5)
Yes	422 (66.5)
Radiation therapy	
No	389 (61)
Yes	246 (39)
Co-medications of interest	
Antibiotics	150 (24)
Glucocorticoids	
Oral glucocorticoids	165 (26)
Inhaled/topical steroids	30 (5)
NSAIDs	63 (10)
Aspirin	109 (17)
Proton pump inhibitors	239 (38)
Metformin	38 (6)
Other oral antidiabetic drug	40 (6)
Insulin	23 (4)
Statins	133 (21)
Fibrates	9 (1)
ACE inhibitors/ARBs	203 (32)
Morphine	130 (20)
Immunosuppressors	4 (0.6)
Hormone replacement therapies	14 (2)
Psychotropic drugs	262 (41)

ACE: angiotensin-converting enzyme; ARBs: angiotensin receptor blockers;

**Table 2. Univariable Cox regression analysis of Overall Survival**

Co-medication	All cancers (n=635)		Melanoma (n=293)		Lung (n=150)		Renal (n=83)	
	Hazard Ratio (95% CI)	<i>p</i>	Hazard Ratio (95% CI)	<i>p</i>	Hazard Ratio (95% CI)	<i>p</i>	Hazard Ratio (95% CI)	<i>p</i>
Antibiotics	1.66 (1.34-2.07)	<b>&lt; .001</b>	1.80 (1.23-2.63)	<b>0.002</b>	1.54 (1.01-2.34)	<b>0.04</b>	1.98 (1.05-3.74)	<b>0.034</b>
Glucocorticoids	1.46 (1.26-1.69)	<b>&lt; .001</b>	1.51 (1.23-1.85)	<b>&lt; .001</b>	1.36 (0.99-1.86)	0.052	2.03 (1.27-3.23)	<b>0.003</b>
Glucocorticoids ≤ 10mg/d	1.12 (0.79-1.59)	0.5	1.47 (0.90-2.40)	0.1	0.65 (0.32-1.32)	0.2	2.27 (0.88-5.89)	0.09
Glucocorticoids 10-20mg/d	2.47 (1.57-3.88)	<b>&lt; .001</b>	2.74 (1.55-4.83)	<b>0.001</b>	1.77 (0.76-4.13)	0.2	-	-
Glucocorticoids > 20mg/d	2.97 (2.33-3.78)	<b>&lt; .001</b>	3.50 (2.38-5.15)	<b>&lt; .001</b>	1.81 (1.18-2.77)	<b>0.006</b>	6.07 (2.72-13.54)	<b>&lt; .001</b>
Protons pump inhibitors	1.70 (1.40-2.08)	<b>&lt; .001</b>	2.18 (1.61-2.96)	<b>&lt; .001</b>	1.42 (0.96-2.09)	0.07	1.09 (0.60-1.99)	0.8
Statins	0.96 (0.75-1.23)	0.7	0.91 (0.62-1.33)	0.6	1.01 (0.65-1.59)	0.9	0.87 (0.43-1.75)	0.7
Morphine	2.69 (2.15-3.38)	<b>&lt; .001</b>	2.73 (1.78-4.21)	<b>&lt; .001</b>	2.52 (1.69-3.76)	<b>&lt; .001</b>	4.16 (2.15-8.07)	<b>&lt; .001</b>
NSAIDs	1.36 (0.99-1.85)	0.05	1.30 (0.80-2.12)	0.3	1.39 (0.72-2.67)	0.3	1.19 (0.55-2.56)	0.7
Aspirin	1.42 (1.11-1.82)	<b>0.005</b>	1.74 (1.16-2.61)	<b>0.008</b>	1.03 (0.67-1.57)	0.9	1.16 (0.56-2.42)	0.7
ACE inhibitors/ARBs	0.98 (0.79-1.21)	0.9	1.24 (0.91-1.69)	0.2	0.78 (0.51-1.19)	0.2	0.74 (0.40-1.36)	0.3
Psychotropic drugs	1.82 (1.49-2.22)	<b>&lt; .001</b>	2.34 (1.73-3.17)	<b>&lt; .001</b>	1.13 (0.77-1.66)	0.5	1.73 (0.94-3.16)	0.08
Metformin	0.98 (0.64-1.50)	0.9	1.40 (0.78-2.52)	0.3	0.73 (0.32-1.67)	0.4	0.68 (0.21-2.19)	0.5
Other oral antidiabetic drugs	0.99 (0.66-1.49)	0.9	1.87 (1.01-3.44)	<b>0.045</b>	0.77 (0.36-1.66)	0.5	1.13 (0.45-2.88)	0.8
Insulin	1.68 (1.01-2.77)	<b>0.043</b>	2.21 (0.91-5.39)	0.08	1.68 (0.53-5.30)	0.4	0.77 (0.19-3.20)	0.7

ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blocker; CI: confidence interval; d: day; NSAIDs: non-steroidal anti-inflammatory drugs

**Table 3. irAE occurrence according to baseline co-medication**

Co-medication	Odds ratio	95% CI	<i>p</i> value
Antibiotics	0.5	0.33-0.76	<b>0.0007</b>
Glucocorticoids	0.63	0.44-0.92	<b>0.01</b>
Protons pump inhibitors	0.53	0.36-0.75	<b>0.0002</b>
Statins	0.9	0.6-1.35	0.6
Morphine	0.26	0.16-0.42	<b>&lt;0.0001</b>
NSAIDs	0.6	0.31-1	<b>0.04</b>
Aspirin	0.63	0.4-0.99	<b>0.037</b>
ACE inhibitors/ARBs	1.09	0.76-1.54	0.6
Psychotropic drugs	0.6	0.43-0.84	<b>0.002</b>
Metformin	0.93	0.45-1.9	0.8
Other oral antidiabetic drugs	0.95	0.47-1.9	0.8
Insulin	0.64	0.21-1.71	0.3