Use of concomitant proton pump inhibitors, statins, or metformin in patients treated with pembrolizumab for advanced urothelial carcinoma: Data from the ARON-2 retrospective study

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Abstract

Background: Concomitant medications may potentially affect the outcome of cancer patients. In this sub-analysis of the ARON-2 real-world study (NCT05290038), we aimed to determine whether the concomitant use of proton pump inhibitors (PPI), statins, or metformin affects outcome of patients with metastatic urothelial cancer (mUC) receiving second-line pembrolizumab.

Patients and Methods: We collected data from the hospital medical records of patients with mUC treated with pembrolizumab as second-line therapy at 87 Institutions from 22 countries. Patients were assessed for overall survival (OS), progression-free survival (PFS), and overall response rate (ORR). We carried out a survival analysis by a Cox regression model.

Results: A total of 802 patients were eligible for this retrospective study; the median follow-up time was 15.3 months. PPI users compared to non-users showed inferior PFS (4.5 *vs*. 7.2 months, p=0.002) and OS (8.7 *vs*. 14.1 months, p<0.001). Concomitant PPI administration remained a significant predictor of PFS and OS after multivariate Cox analysis. The use of statins or metformin was not associated with response or survival.

Conclusions: Our study results suggest a significant prognostic impact of concomitant PPI use in mUC patients receiving pembrolizumab in the real-world context. The mechanism of this interaction warrants further elucidation.

Keywords: Urothelial Cancer; Proton pump inhibitors; Statins; Metformin; ARON-2 study; Clinical trial; Drug-drug interactions; Immunotherapy; NCT05290038.

Introduction

In recent years, the introduction of immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein-1 (PD-1)/ligand-1 (PD-L1) for the treatment of advanced or metastatic urothelial carcinoma (mUC) has improved outcomes and quality of life of these patients [1,2]. Currently, ICIs are approved in three indications for mUC: 1) first-line treatment in cisplatin-ineligible patients with high PD-L1 expressing tumors or any platinum-ineligible patients regardless of PD-L1 status (atezolizumab, pembrolizumab); 2) second-line treatment following platinum-based chemotherapy or progression within 12 months after neoadjuvant or adjuvant chemotherapy (pembrolizumab, nivolumab, atezolizumab), and 3) switch maintenance therapy following platinum-based chemotherapy (avelumab) [2-5].

Patients with advanced solid cancers often have other relevant comorbidities, implying a significant number of concomitant medications. Drug-drug interactions as well as potential anticancer effects of commonly used drugs have become an area of increasing interest. Proton pump inhibitors (PPIs), statins, and metformin represent the most commonly prescribed drugs worldwide including cancer patients. Recent studies have reported possible detrimental effects of concomitant medications, including antibiotics, PPIs, and corticosteroids, on the efficacy of ICIs in patients with several malignancies [6-16], including mUC [13-17]. In particular, PPIs are among the most commonly prescribed drugs for patients with cancer to reduce gastrointestinal toxicity associated with certain anticancer drugs [18]. However, the potential risk of their direct and/or indirect interactions with anticancer agents is high in terms of bioavailability, pharmacokinetics, and immunological interference, the latter possibly mediated by microbiota [19-20]. Consequently, the effectiveness of anticancer compounds may be altered by taking concomitant PPI alone or in combination with steroids and/or antibiotics [7, 16, 21]. The impact of PPI intake during ICI immunotherapy for mUC has been suggested very recently

by three relatively small studies [13-17]. In contrast to PPIs, the concomitant administration of metformin or statins seems to be associated with favorable outcome in patients with advanced cancer treated with ICIs. Recent data suggest potential synergistic antitumor effects of the concomitant use of ICIs as monotherapy with the oral hypoglycemic agent metformin in patients with cancer and diabetes mellitus, possibly due to the elicitation of multiple cross-mechanisms between cancer metabolism and the host immune system in controlling cancer cell growth [22]. Nevertheless, when ICIs were used in the context of the first-line combination therapy in patients with advanced renal cell carcinoma, the role of concomitant metformin intake was not confirmed [23]. Additionally, concomitant statin exposure in cancer patients treated with ICIs may also lead to favorable outcomes. It has been demonstrated that statins exert a series of biological activities with high potential to enhance the effect of ICIs. Specifically, statins modulate the cancer cell metabolism and are also involved in multiple immune system functions including T-cell signaling, antigen presentation, immune cell migration and cytokine production [23-26]. To our knowledge, there are no data on a possible association between metformin or statin intake and outcome of mUC patients treated with ICIs.

The ARON project has been designed to create a global network to allow uro-oncologists to share and discuss their experiences on the use of immunotherapy and other emerging drugs in patients with genitourinary cancers. Specifically, the ARON-2 study (NCT05290038) was designed to globally collect real-world data on the use of pembrolizumab for mUC. The aim of the present analysis was to assess the association of concomitant use of PPIs, metformin, or statins with patient outcomes in the ARON-2 study population.

Patients and Methods

Study design and patient population

We retrospectively analyzed data from patients with a cytologically and/or histologically

confirmed mUC, treated with second-line pembrolizumab. The clinical data were collected between January 1, 2016 and October 1, 2022. We retrospectively reviewed anonymized data obtained from the hospital information systems sent by participating centres.

The study protocol "ARON-2" (No. 2022 39) was approved by the Ethical Committee of the Marche Region (Italy) on February 17, 2022, and complied with the International Ethical Guidelines for Biomedical Research, the Declaration of Helsinki, and local laws in each participating center. The Informed consent with subsequent analysis of the follow-up data was obtained from all the participants.

Pembrolizumab was administered intravenously as a single agent at the standard approved schedule (200 mg every 3 weeks). The treatment was continued until disease progression, unacceptable toxicity, or patient refusal. None of the patients had received prior ICI therapy. Concomitant PPIs, statins, and metformin were administered orally at individualized doses under the supervision the patients' healthcare providers. Standard follow-up for patients receiving pembrolizumab generally consisted of periodic physical examinations and laboratory analyses were usually carried out every 3–6 weeks. Computed tomography (CT) or magnetic resonance (MR) was performed at baseline and every 2–4 months thereafter, according to physicians' practice, or when disease progression was clinically suspected.

Study endpoints

The primary endpoint of the ARON-2 study was response, assessed by progression-free survival (PFS), overall survival (OS) and overall response rate (ORR). This sub-analysis evaluates the correlation between the concomitant use of PPIs, metformin or statins and these outcomes of mUC patients treated with pembrolizumab. PFS was defined as the time from the start of pembrolizumab to progression or death from any cause, whichever

occurred first. OS was defined as the time from the start of pembrolizumab until death or lost at follow-up. We considered as censored those patients without progression or death at the last follow-up. The objective response to pembrolizumab was assessed according to RECIST 1.1 principles and data on complete (CR) or partial responses (PR), stable disease (SD) or progressive disease (PD) were collected and analyzed [27]. ORR was defined as the proportion of patients who achieved CR or PR per RECIST 1.1 criteria.

Statistical analysis

PFS and OS were estimated by using the Kaplan-Meier method with Rothman's 95% confidence intervals (CI) and compared using the log-rank test. The median follow-up was calculated with the Kaplan–Meier method. Univariate and multivariate analyses were performed by using Cox proportional hazards models. The chi-square test was used to assess potential differences between PPIs, statin or metformin users *vs*. non-users in terms of patient characteristics and ORR. Significance levels were set at a 0.05 value and all *p* values were two-sided. The statistical analysis was performed by using MedCalc version 19.6.4 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium).

Results

Baseline characteristics

In total, the study included 802 mUC patients. The baseline patient characteristics are summarized in **Table 1**. The median follow-up time was 15.3 months (95%CI 14.0−76.0). In 307 patients (38%), pembrolizumab therapy was ongoing at the time of data cut-off; 430 patients (54%) had died at the time of data cut-off; 535 patients (67%) received pembrolizumab following progression during first-line platinum-based chemotherapy (**Cohort A**) and 267 (33%) after disease recurrence within <12 months since the completion of adjuvant or neoadjuvant chemotherapy (**Cohort B**). One hundred and sixty-two (32.7%)

of the 495 patients with progression on pembrolizumab received further therapies (103 in cohort A and 59 in cohort B).

Concomitant use of PPIs, statins or metformin was reported in 372 (46%), 185 (23%) and 98 (12%) patients, respectively. The use of metformin was significantly more frequent in patients aged ≥65y, while no significant differences were found in baseline patient characteristics according to the concomitant use of PPIs or statins (**Table 1**).

Survival analysis

Median PFS and OS for the whole cohort were 6.2 (95% CI: 5.1−6.9) and 11.3 months (95% CI: 9.5−13.0), respectively.

Median PFS and OS for PPI users were 4.5 (95% CI: 3.7−6.3) and 8.7 (95% CI: 6.9−11.4) months *vs*. 7.2 (95% CI: 5.7−9.1) and 14.1 months (95% CI: 10.4−16.2) for PPI non-users (p=0.002 and p<0.001, respectively) (**Figure 1**).

Median PFS and OS for statin users were 6.9 (95% CI: 4.0−9.5) and 10.3 (95%CI: 8.0−15.3) months *vs*. 6.0 (95%CI: 4.8−6.9) and 11.3 months (95% CI: 9.1−13.4) months for statin non-users (p=0.715 and p=0.999, respectively) (**Figure 1**).

Median PFS and OS for metformin users were 7.1 (95% CI 3.7−12.0) and 12.4 (95% CI: 7.8−16.0) months *vs*. 6.2 (95% CI 5.0−6.9) and 10.5 (95%CI: 9.0−13.3) months for metformin non-users (p=0.630 and p=0.896, respectively) (**Figure 1**).

In the Cox multivariate analysis, the use of PPIs remains a significant and independent factor predicting both PFS (HR=1.28, 95% CI 1.08−1.53, p=0.006) and OS (HR=1.41, 95% CI 1.17−1.71, p≤0.001). Other independent prognostic factors were synchronous metastatic disease, bone and liver metastases for PFS and smoking status, synchronous metastatic disease, bone and liver metastases for OS (**Table 2**).

Subgroup survival analyses according to the concomitant use of PPIs

The association of concomitant use of PPIs with shorter OS was statistically significant in both males (p=0.004) and females (p=0.019) (**Figure 2**); patients aged ≥65y (p=0.001) (**Figure 2**); current or former smokers (p<0.001) (**Figure 2**); patients with ECOG-PS 0-1 (p=0.004); patients with pure UC histology (p=0.002) and those with other histological variants (p=0.034) (**Figure 3**); patients with UC of lower urinary tract (p=0.001) (**Figure 3**); patients with metachronous metastatic disease (p=0.001); patients with non-regional lymph node metastases (p<0.001), patients with bone metastases (p=0.012) (**Figure 3**); and patients in cohort A (p<0.001) (**Figure 3**). The difference in OS according to the use of PPIs was not statistically significant in patients aged <65y (p=0.135); non-smokers (p=0.057); patients with ECOG-PS≥2 (p=0.150); patients with upper urinary tract tumors (p=0.112); patients with lung metastases (p=0.179); patients with liver metastases (p=0.122); patients with synchronous metastatic disease (p=0.067); and patients in cohort B (p=0.262).

Similar to OS, the association of concomitant use of PPIs with shorter PFS was statistically significant in both males (p=0.020) and females (p=0.031) (**Figure 4**); patients aged ≥65y (p=0.013) and those aged <65y (p=0.05) (**Figure 4**); current or former smokers (p=0.006) (**Figure 4**); patients with ECOG-PS 0-1 (p=0.017); patients with pure UC histology (p=0.006) (**Figure 4**); patients with UC of lower urinary tract (p<0.001) (**Figure 4**); patients with metachronous (p=0.025) and those with synchronous metastatic disease (p=0.016); patients with non-regional lymph node metastases (p<0.001) (**Figure 5**); and patients in cohort A (p=0.008) (**Figure 5**). The difference in PFS according to the use of PPIs was not statistically significant in non-smokers (p=0.12); patients with other histological UC variants (p=0.120); patients with ECOG-PS ≥2 (p=0.179); patients with upper urinary tract tumors (p=0.595); patients with bone metastases (p=0.317); lung metastases (p=0.625); patients with liver metastases (p=0.550) and those in cohort B (p=0.150).

The survival data are summarized in detail in the **Table 3**.

Objective response

Eighty patients (10%) achieved CR, 168 (21%) PR, 197 (24%) SD and 357 (44%) PD, with an ORR of 31%. The OS was significantly different according to the type of response: NR (95%CI NR−NR), 34.4 months (95%CI 22.4−47.2), 15.6 months (95%CI 12.4−19.4) and 4.3 months (95%CI 3.8−30.4) in patients with CR, PR, SD, and PD, respectively (p<0.001).

Stratified by concomitant medications, the ORR was 26% in PPI users and 36% in PPI non-users (CR=8%, PR=18%, SD=24%, PD=50% vs CR=12%, PR=24%, SD=24%, PD=40% - p=0.127). No difference was found in terms of ORR between statin users *vs*. non-users (ORR=35% - CR=12%, PR=23%, SD=22%, PD=43% - *vs*. ORR=29% - CR=9%, PR=20%, SD=26%, PD=45% - p=0.364) or between metformin users *vs*. non-users (ORR=33%, CR=11%, PR=22%, SD=24%, PD=43%, vs. ORR=31%, CR=10%, PR=21%, SD=25%, PD=44%, p=0.762).

Discussion

In the last decade, immunotherapy has increasingly gained a key role in the treatment of several solid tumors and hematological malignancies. In addition, ICIs demonstrated a certain efficacy in various setting in solid tumors: neoadjuvant, adjuvant, first-line or successive lines of therapy. A perfect example of this expanding use of immunotherapeutic agents represents advanced UC. Since the initial second-line strategy approval for patients who progressed on a prior platinum-based chemotherapy, the use of ICIs in mUC has been quickly extended to the first-line setting in PD-L1 positive cisplatin-ineligible patients or any platinum-ineligible patients regardless of PD-L1 expression as well as to the switch maintenance therapy in platinum-responders [28].

As we witness this indisputable therapeutic revolution, an emerging and important issue that remains to be solved is whether the concomitant administration of other drugs may

impair or eventually enhance the efficacy of ICIs. This is a crucial question also considering that UC mostly affects elderly patients who may present other disorders and use several concomitant medications.

In our study, the concomitant use of PPIs was significantly associated with both shorter PFS and OS in mUC patients receiving pembrolizumab therapy. Furthermore, its independent adverse prognostic role was confirmed in the multivariate analysis. The use of statins or metformin did not affect the response or survival outcomes of these patients.

PPIs, statins and metformin represent the most commonly prescribed medications in the global population; however, their potential to affect the efficacy of ICIs in cancer patients has not been fully elucidated.

The ability of PPIs to influence the efficacy of ICIs may be mainly related to the modifications induced by PPIs in gut microbiome, which plays a crucial role in patients treated with ICIs for solid tumors [29]. It has been shown that PPI use can induce marked alterations to the intestinal microbiome including changes in composition, reduction of the alpha diversity, small intestinal bacterial overgrowth and manifestation of oral bacteria in more distal parts of the intestine [30−32]. In this regard, Imhann *et al.* [33] observed that PPI use is associated with the presence of multiple oral bacteria over-represented in the faecal microbiome, with an overall significant increase in bacteria, including Enterococcus, Streptococcus, Staphylococcus and Escherichia coli. There has been an accumulating body of evidence that the concomitant use of PPIs could have detrimental effect on ICI therapy in various cancer types [16−18]. In paticular, this issue has been underexplored in the field of mUC. Several retrospective studies have been reported recently. However, their crucial limitation is based on a relatively small number of included patients, especially those of PPI users, potentialy introducing a significant bias. Thus, there has been a lack for solid data from large studies. The association of shorter PFS and OS with concomitant use of PPIs (HR: 1.70, 95% CI: 1.23-2.35, p=0.001 and HR: 2.02, 95% CI:

1.28-3.18, *p*=0.003, respectively) in a cohort of 227 mUC patients treated with pembrolizumab has been found in a retrospective study conducted by Fukuokaya *et al.* [31]. Similar data were reported two other studies conducted by Tomisaki *et al.* and Okuyama *et al.* including 40 and 155 patients, respectively. These results are in agreement with our large study including 802 patients (372 PPI users). Aside from mUC patients treated with pembrolizumab, similar findings were found in a *post hoc* analysis of IMvigor210 and IMvigor211 clinical trials conducted by Hopkins *et al.* [9]. They found that PPI use was associated with shorter PFS and OS (HR: 1.38, 95% CI: 1.18-1.62, *p*<0.001 and HR: 1.52, 95% CI: 1.27-1.83, *p*<0.001, respectively) in advanced-stage UC patients treated with atezolizumab, while there was no association in those treated with chemotherapy [9].

On the other hand, a study by Kunimitsu *et al.* including 79 patients failed to show such a prognostic role of the use of PPIs in advanced-stage UC when treated with pembrolizumab [15]. Nonetheless, it is another study fundamentally limited by a small cohort size.

Notably, our data show that the use of PPIs was significantly associated with poor outcome only in patients with primary tumors of the lower urinary tract but not in those with primary tumors of the upper urinary tract. This may be explained by a relatively small number and the overall poor prognosis of mUC patients with tumors originating in the upper urinary tract and their generally reduced benefit from ICIs [34] and a distinct genomic background of this specific subgroup [35]. However, furher elucidation of this issue is needed.

Anticancer properties of statins such as the inhibition of tumor cell growth, invasion and metastatic potential have been suggested in various experimental studies [36−38]. Notwithstanding, clinical studies evaluating the impact of statin use in cancer patients including those with UC have been conducted with inconclusive results. Moreover, there are no data on the role of the statin use in mUC patients treated with ICIs. Ferro *et al.*

suggested that statins may have a beneficial effect on recurrence rates in patients with high grade non-muscle invasive urothelial bladder cancer in a multicenter study including 1510 patients [39]. Oppositely, another recent retrospective study by Haimerl *et al*. found no impact of statin use on bladder cancer recurrence or survival in a cohort of 972 patients [40]. In our study we found no impact of the concomitant statin use in mUC patients treated with pembrolizumab.

Metformin represents another commonly used drug with suggested anticancer activity which is related to both, direct effects on cancer cells based on inhibition of cancer-related signaling pathways and indirect effects on the host based on lowering blood glucose and insulin as well as anti-inflammatory effects [41,42]. Although several retrospective studies show the association of metformin use with favorable prognosis of patients with cancer, there are no data on its role in patients with mUC treated with ICIs. Our data show no impact of the concomitant metformin use in mUC patients treated with pembrolizumab.

Our study presents several limitations, mainly due to its retrospective nature. A centralized review of radiological imaging was not performed. The dosage and duration of the investigated comedication exposure could not be assessed from the available data sources. Furthermore, we had no available data on other concomitant medications (i.e. steroids, antibiotics) or patients' comorbidities that could affect the efficacy of pembrolizumab. Consequently, our results should be interpreted with caution and are in need of a further prospective validation. On the other hand, the major strength of our study is based on a large patient population, which allowed us to perform a detailed subgroup analysis.

Conclusion

Our data show that the concomitant use of PPIs may adversely affect the outcome of mUC patients treated with second-line pembrolizumab. Further studies investigating the biological and immunological background of this interaction are warranted in order to optimize the outcome of patients receiving immunotherapy in this setting.

Conflicts of Interest

O. Fiala received honoraria from Roche, Janssen, GSK and Pfizer for consultations and lectures unrelated to this project. S. Buti received honoraria as speaker at scientific events and advisory role by BMS, Pfizer, MSD, Ipsen, AstraZeneca, Merck, all unrelated to this project. M. Santoni has received research support and honoraria from Janssen, Bristol Myers Squibb, Ipsen, MSD,Astellas and Bayer, all unrelated to this project. R. Kanesvaran has received fees for speaker bureau and advisory board activities from the following companies; Pfizer, MSD, BMS, Eisai, Ipsen, Johnson and Johnson, Merck, Amgen, Astellas and Bayer, all unrelated to this project. E. Grande has received honoraria for speaker engagements, advisory roles or funding of continuous medical education from Adacap, AMGEN, Angelini, Astellas, Astra Zeneca, Bayer, Blueprint, Bristol Myers Squibb, Caris Life Sciences, Celgene, Clovis-Oncology, Eisai, Eusa Pharma, Genetracer, Guardant Health, HRA-Pharma, IPSEN, ITM-Radiopharma, Janssen, Lexicon, Lilly, Merck KGaA, MSD, Nanostring Technologies, Natera, Novartis, ONCODNA (Biosequence), Palex, Pharmamar, Pierre Fabre, Pfizer, Roche, Sanofi-Genzyme, Servier, Taiho, and Thermo Fisher Scientific and has received research grants from Pfizer, Astra Zeneca, Astellas, and Lexicon Pharmaceuticals, all unrelated to this project. F. S. M. Monteiro has received research support from Janssen, Merck Sharp Dome and honoraria from Janssen, Ipsen, Bristol Myers Squibb and Merck Sharp Dome, all unrelated to this project. C. Porta has received honoraria from Angelini Pharma, Astra Zeneca, BMS, Eisai, General Electric, Ipsen and MSD and acted as a Protocol Steering Committee Member for BMS, Eisai and MSD, all

unrelated to this project. Z. Myint has received research support from Merck unrelated to this project. J. Molina-Cerrillo declares consultant, advisory or speaker roles for IPSEN, Roche, Pfizer, Sanofi, Janssen, and BMS and has received research grants from Pfizer, IPSEN and Roche, all unrelated to this project. P. Giannatempo has received research support from Ipsen, Astra Zeneca, MSD and honoraria for speaker engagements, advisory roles from Astellas, MSD, Janssen, Pfizer, all unrelated to this project. E. T. Lam has received institutional research funding from Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, Bristol-Myers Squibb, Pfizer, and F. Hoffmann-La Roche Ltd.
The other authors declare to have no conflicts of interest.

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Patients	Overall 802 (%)	PPI users 372 (%)	PPI non-users 430 (%)	*p*	Statin users 185 (%)	Statin non-users 617 (%)	*p*	Metformin users 98 (%)	Metformin non-users 704 (%)	*p*
Gender										
Male	591 (74)	277 (74)	314 (73)	0.873	145 (78)	446 (72)	0.328	77 (79)	514 (73)	0.322
Female	211 (26)	95 (26)	116 (27)		40 (22)	171 (28)		21 (21)	190 (27)	
Age ≥65y	595 (74)	285 (77)	310 (72)	0.418	151 (81)	444 (72)	0.134	83 (85)	512 (73)	**0.038**
ECOG-PS ≥2	138 (17)	74 (20)	64 (15)	0.353	40 (22)	98 (16)	0.281	13 (13)	125 (18)	0.330
Current or former smokers	521 (65)	253 (68)	268 (62)	0.375	130 (70)	391 (63)	0.296	63 (64)	458 (65)	0.883
Primary tumor location										
Upper urinary tract	214 (27)	108 (29)	106 (25)	0.525	44 (24)	170 (28)	0.520	23 (23)	191 (27)	0.515
Lower urinary tract	588 (73)	264 (71)	324 (75)		141 (76)	447 (72)		75 (77)	513 (73)	
Tumor histology										
Pure urothelial carcinoma	648 (81)	307 (83)	341 (79)	0.472	157 (85)	491 (80)	0.353	87 (89)	561 (80)	0.079
Minor or mixed variants	154 (19)	65 (17)	89 (21)		28 (15)	126 (20)		11 (11)	143 (20)	
Synchronous metastastic disease	246 (31)	113 (30)	133 (31)	0.878	50 (27)	196 (32)	0.439	29 (30)	217 (31)	0.878
Common sites of metastasis	564 (70)	263 (71)	301 (70)		128 (69)	436 (71)				
Lymph nodes (non-regional)	265 (33)	120 (32)	145 (34)	0.877	64 (35)	201 (33)	0.758	73 (74)	491 (70)	0.530
Lung	227 (28)	114 (31)	113 (26)	0.764	52 (28)	175 (28)	0.766	43 (44)	222 (32)	0.081
Bone	146 (18)	71 (19)	75 (17)	0.435	48 (26)	98 (16)	1.000	33 (34)	194 (28)	0.360
Liver	11 (1)	7 (2)	4 (1)	0.714	5 (3)	6 (1)	0.083	22 (22)	124 (18)	0.481
Brain				0.562			0.314	4 (4)	7 (1)	0.175
Pembrolizumab setting										
Cohort A (progressed after first-line chemotherapy)	535 (67)	265 (71)	270 (63)	0.230	115 (62)	420 (68)	0.375	68 (69)	467 (66)	0.651
Cohort B (recurred within <1y from adjuvant/neoadjuvant therapy)	267 (33)	107 (29)	160 (37)		70 (38)	197 (32)		30 (31)	237 (34)	

Table 1: Patients' characteristics in the overall study population and stratified by concomitant medications.

Overall survival	Univariate Cox regression		Multivariate Cox regression	
	HR (95%CI)	*p-value*	HR (95%CI)	*p-value*
Gender (females vs. males)	1.22 (0.99–1.50)	0.062		
Age (≥65y vs. <65y)	1.07 (0.86–1.33)	0.530		
Smoking (smokers vs. non-smokers)	0.81 (0.66–0.98)	**0.030**	0.78 (0.64–0.95)	**0.012**
Histology (mixed vs. pure UC)	1.04 (0.81–1.32)	0.775		
Upper vs. Lower urinary tract	1.10 (0.90–1.36)	0.353		
Synchronous metastatic disease (yes vs. no)	1.32 (1.08–1.61)	**0.007**	1.33 (1.09–1.63)	**0.005**
Lymph node metastases (Y vs. N)	0.86 (0.70–1.06)	0.151		
Bone metastases (Y vs. N)	1.53 (1.25–1.88)	**<0.001**	1.51 (1.22–1.84)	**<0.001**
Liver metastases (Y vs. N)	1.46 (1.16–1.84)	**0.001**	1.39 (1.10–1.75)	**0.006**
Proton Pump Inhibitors (Y vs. N)	1.41 (1.17–1.71)	**<0.001**	1.41 (1.17–1.70)	**<0.001**
Statins (Y vs. N)	1.00 (0.80–1.25)	0.999		
Metformin (Y vs. N)	0.98 (0.73–1.31)	0.896		
Progression-free survival	**Univariate Cox regression**		**Multivariate Cox regression**	
	HR (95%CI)	***p-value***		**HR (95%CI)**
Gender (females vs. males)	0.99 (0.81–1.22)	0.957		
Age (≥65y vs. <65y)	0.93 (0.76–1.14)	0.485		
Smoking (smokers vs. no-smokers)	0.93 (0.77–1.11)	0.409		
Histology (mixed vs. pure UC)	1.03 (0.83–1.29)	0.771		
Upper vs. Lower urinary tract	1.04 (0.86–1.27)	0.681		
Synchronous metastatic disease (yes vs. no)	1.28 (1.06–1.55)	**0.009**	1.24 (1.02–1.49)	**0.027**
Lymph node metastases (Y vs. N)	0.86 (0.71–1.04)	0.130		
Bone metastases (Y vs. N)	1.48 (1.22–1.79)	**<0.001**	1.42 (1.17–1.71)	**<0.001**
Liver metastases (Y vs. N)	1.51 (1.22–1.86)	**<0.001**	1.44 (1.17–1.79)	**<0.001**
Proton Pump Inhibitors (Y vs. N)	1.32 (1.10–1.58)	**0.002**	1.28 (1.08–1.53)	**0.006**

Statins (Y vs. N)	0.96 (0.78−1.19)	0.716		
Metformin (Y vs. N)	0.94 (0.71−1.23)	0.935		
ECOG-PS = Eastern Cooperative Oncology Group-Performance Status; UC = Urothelial Carcinoma				

Table 2: Univariate and multivariate Cox analyses for overall survival (OS) and progression-free survival (PFS).

	Median OS (95% CI)		***p*-value**	**Median PFS (95% CI)**		***p*-value**
	PPI users	**PPI non-users**		**PPI users**	**PPI non-users**	
Whole cohort	8.7 months (6.9−11.4)	14.1 months (10.4−16.2)	***p*<0.001**	4.5 months (3.7−6.3)	7.2 months (5.7−9.1)	***p*=0.002**
Gender						
Males	10.0 months (7.2−13.0)	15.1 months (11.1−17.0)	***p*=0.004**	5.0 months (3.8−6.4)	6.9 months (5.6−9.1)	***p*=0.020**
Females	6.3 months (4.6−46.4)	11.3 months (7.5−18.6)	***p*=0.019**	4.0 months (3.2−44.0)	8.3 months (4.6−12.4)	***p*=0.031**
Age						
< 65y	9.7 months (5.-16.8)	12.6 months 9.2-22.4)	*p*=0.135	4.2 months (3.4-44.0)	6.9 months (4.7-9.5)	***p*=0.05**
≥ 65y	8.7 months (6.4−11.4)	14.1 months (10.0−17.0)	***p*=0.001**	4.6 months (3.6−6.4)	7.5 months (5.6−10.0)	***p*=0.013**
Smoking						
Current or former smokers	10.0 months (6.9−12.4)	16.0 months (12.4−22.4)	***p*<0.001**	4.5 months (3.7−6.9)	7.5 months (6.1−11.4)	***p*=0.006**
Non-smokers	7.4 months (4.6-12.2)	10.2 months (7.5-14.1)	*p*=0.057	4.5 months (3.4-6.6)	6.0 months (4.2-9.5)	*p*=0.12
Histology						
Pure UC	8.9 months (7.0−11.5)	14.3 months (10.4−16.5)	***p*=0.002**	5.3 months (3.7−6.6)	7.2 months (5.7−9.4)	***p*=0.006**
Other variants	6.4 months (3.6−46.4)	12.4 months (8.3−23.4)	***p*=0.034**	3.9 months (3.2−44.0)	7.0 months (4.7−20.9)	*p*=0.120
ECOG-PS						

0-1	11.5 months (8.9-14.9)	16.2 months (13.3-22.2)	***p*=0.004**	6.4 months (4.5-7.3)	8.5 months (6.7-10.4)	***p*=0.017**
≥ 2	3.2 months (2.6−4.1)	5.1 months (4.1−6.6)	*p*=0.150	2.6 months (1.9−3.3)	3.5 months (2.5−4.8)	*p*=0.179
Primary tumor site						
Lower urinary tract	9.7 months (7−11.7)	14.6 months (11.1−17.5)	***p*=0.001**	4.5 months (3.7−6.3)	7.7 months (6.2−9.9)	***p*<0.001**
Upper urinary tract	11.8 months (8.3−16.2)	7.2 months (4.8−12.1)	*p*=0.112	5.3 months (3.4−8.0)	5.4 months (4.1−9.5)	*p*=0.595
Type of metastatic spread						
Synchronous metastases	6.2 months (4.0−9.7)	10.0 months (7.4−47.2)	*p*=0.067	3.6 months (2.6−5.8)	6.6 months (3.9−9.5)	***p*=0.016**
Metachronous metastases	10.2 months (7.8-13.0)	15.8 months (12.5-22.2)	***p*=0.001**	6.1 months (4.0-7.2)	7.7 months (5.8-9.9)	***p*=0.025**
Site of distant metastases						
Lymph node (non-regional)	8.7 months (6.4−11.7)	15.4 months (12.5−19.0)	***p*<0.001**	4.5 months (3.5−6.3)	8.6 months (6.4−12.4)	***p*<0.001**
Bone	5.8 months (3.9−7.0)	6.8 months (5.5−16.2)	***p*=0.012**	3.3 months (2.9−4.6)	3.8 months (3.2−5.6)	*p*=0.317
Lung	8.1 months (5.8−11.7)	11.3 months (7.4−15.5)	*p*=0.179	6.2 months (3.5−7.2)	5.5 months (4.1−7.7)	*p*=0.625
Liver	4.5 months (3.6−8.1)	10.0 months (6.7−16.0)	*p*=0.122	3.6 months (2.5−4.5)	3.9 months (3.3−4.9)	*p*=0.550
Pembrolizumab setting						
Cohort A (progressed after first-line chemotherapy)	7.2 months (5.9−9.7)	12.6 months (9.2−15.8)	***p*<0.001**	4.3 months (3.5−6.1)	6.4 months (4.8−8.4)	***p*=0.008**
Cohort B (recurred within <1y from adjuvant/neoadjuvant therapy)	16.0 months (8.8−21.2)	17.0 months (10.3−45.9)	*p*=0.262	6.9 months (3.5−14.9)	8.6 months (6.2−25.4)	*p*=0.150

ECOG-PS = Eastern Cooperative Oncology Group-Performance Status; UC = Urothelial Carcinoma, CI = Cinfidence interval, y = years; statistically significant *p*-values are in bold

Table 3: Summary of patient survival data according to the specific subgroups

Figure Legends

Figure 1. Overall survival (OS) and progression-free survival (PFS) according to the use of concomitant proton pump inhibitors (PPIs), statins or metformin.

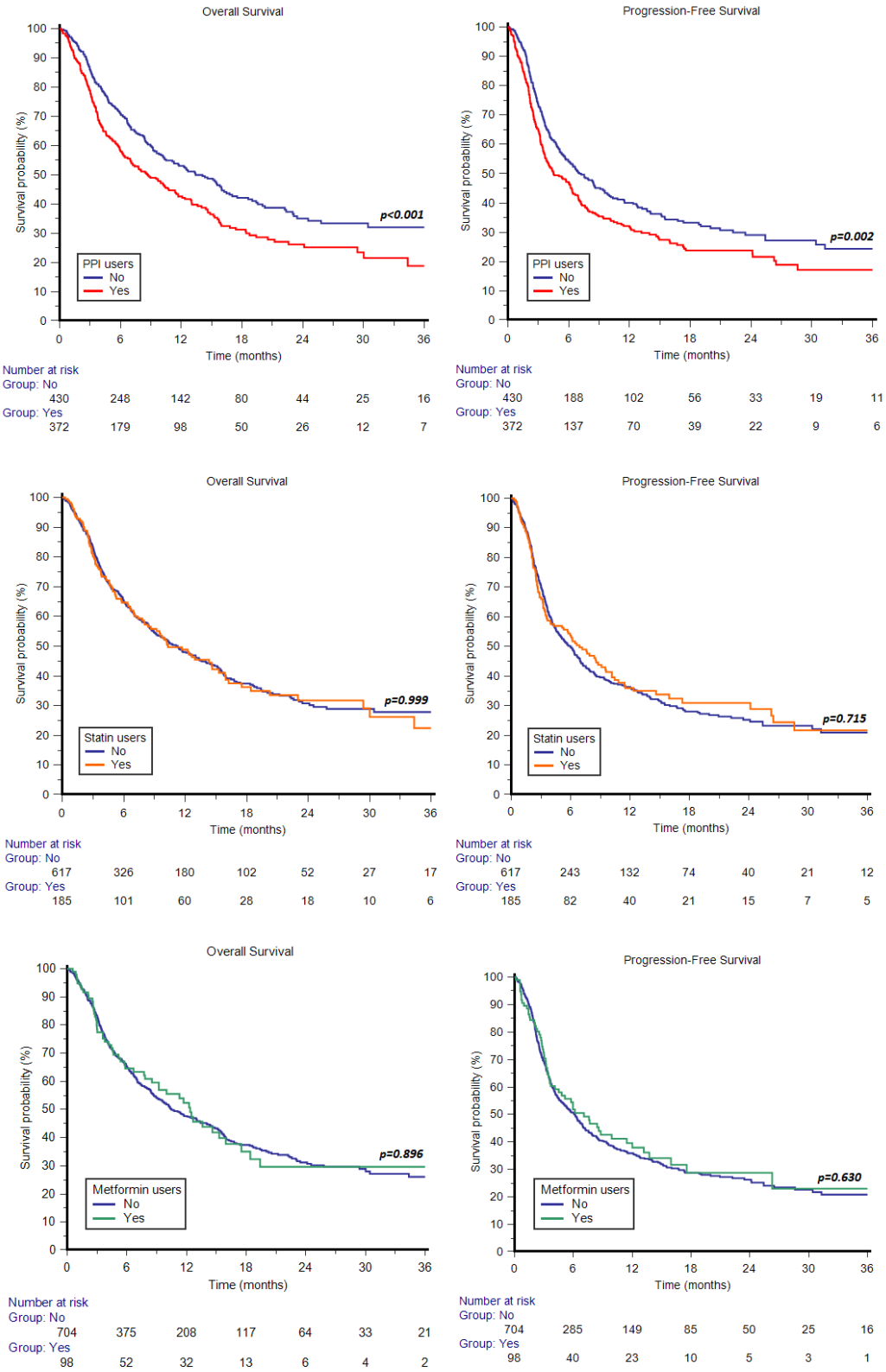


Figure 2. Overall survival according to concomitant use of PPIs stratified by sex, smoking status and age.

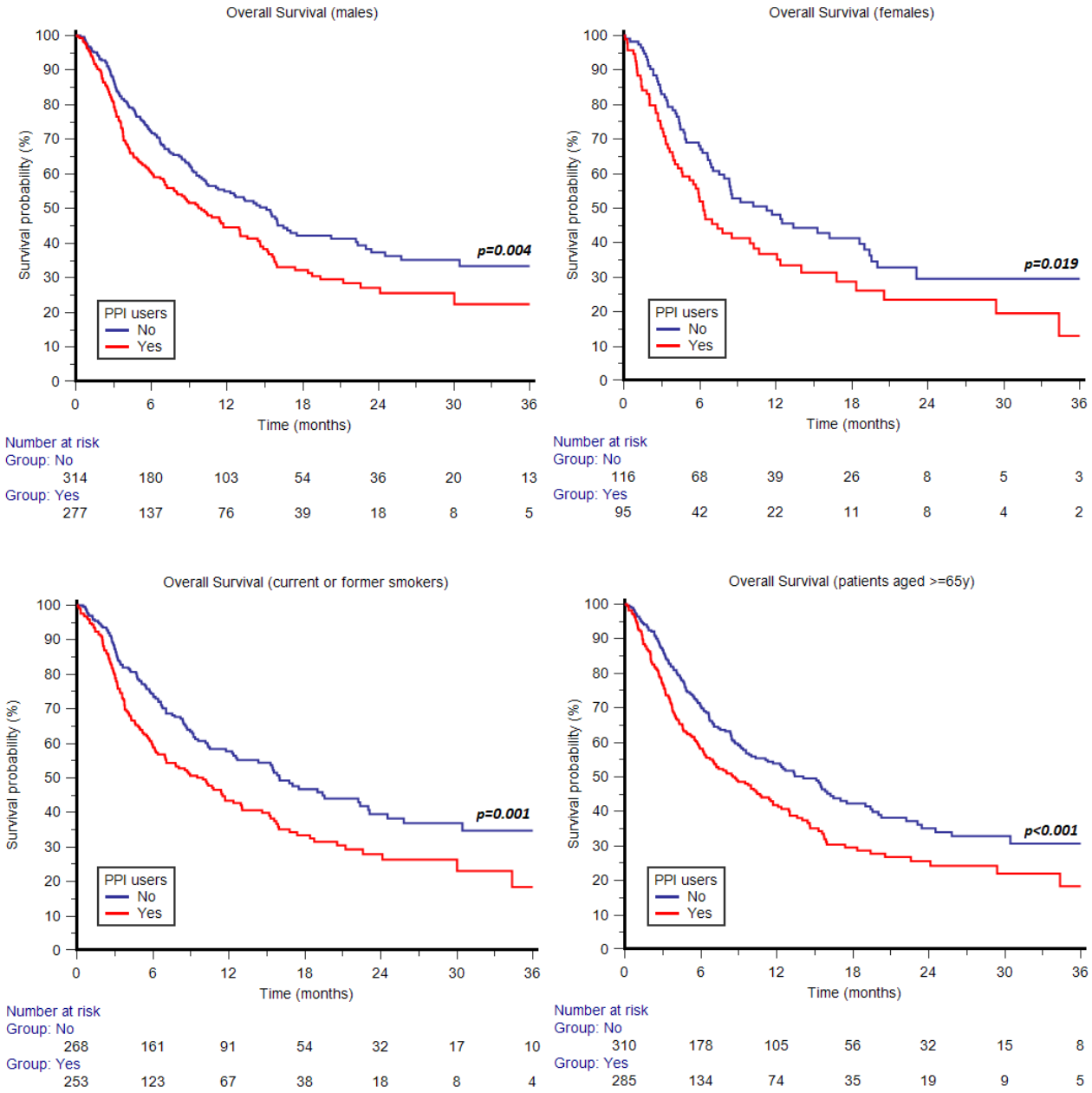


Figure 3. Overall survival according to concomitant use of PPIs stratified by tumor histology, primary tumor site, lymph node or bone metastases and pembrolizumab setting (second-line therapy after progression on first-line platinum-based chemotherapy, Cohort A)

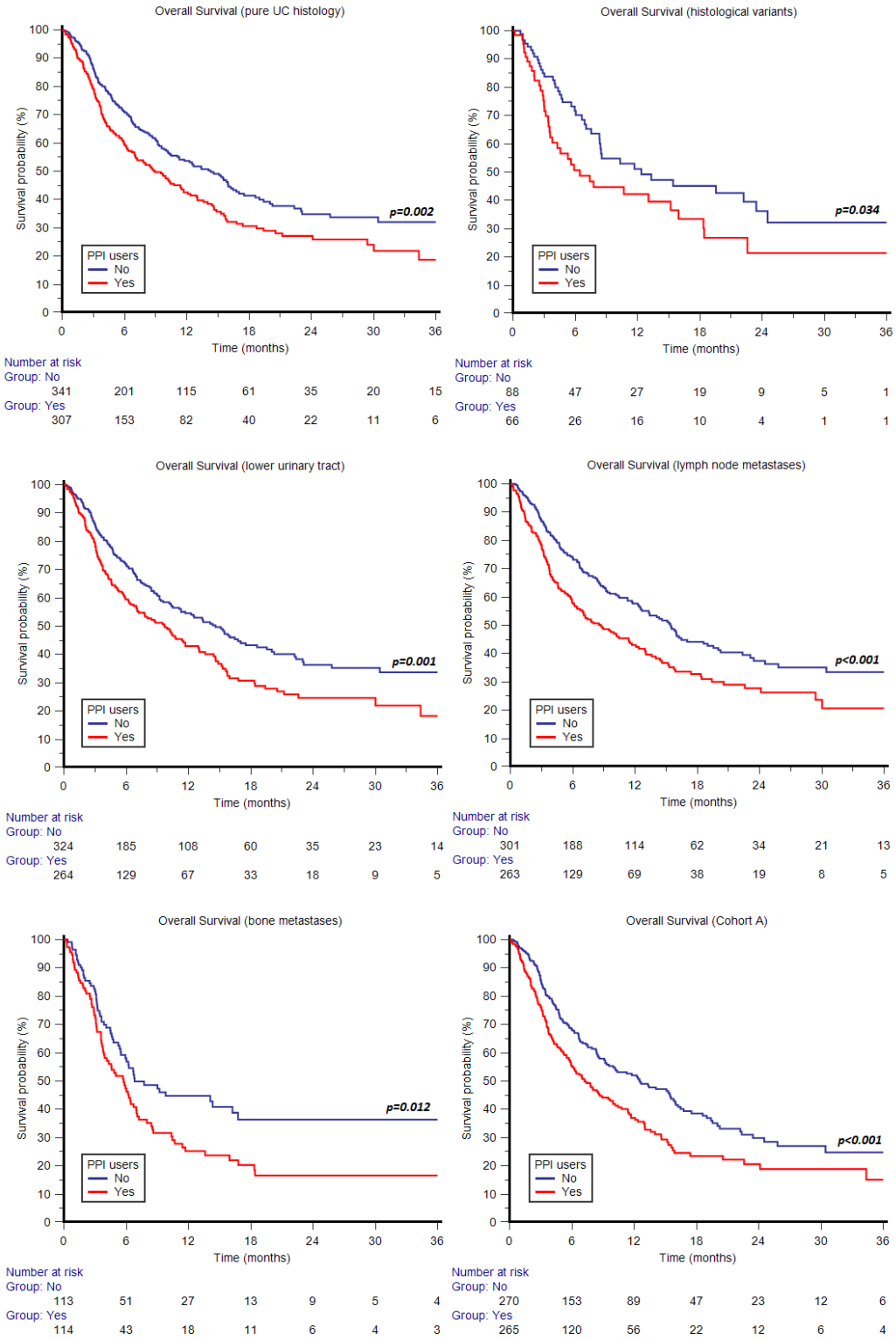


Figure 4. Progression-free survival according to concomitant use of PPIs stratified by sex, age, smoking status, tumor histology and primary tumor site

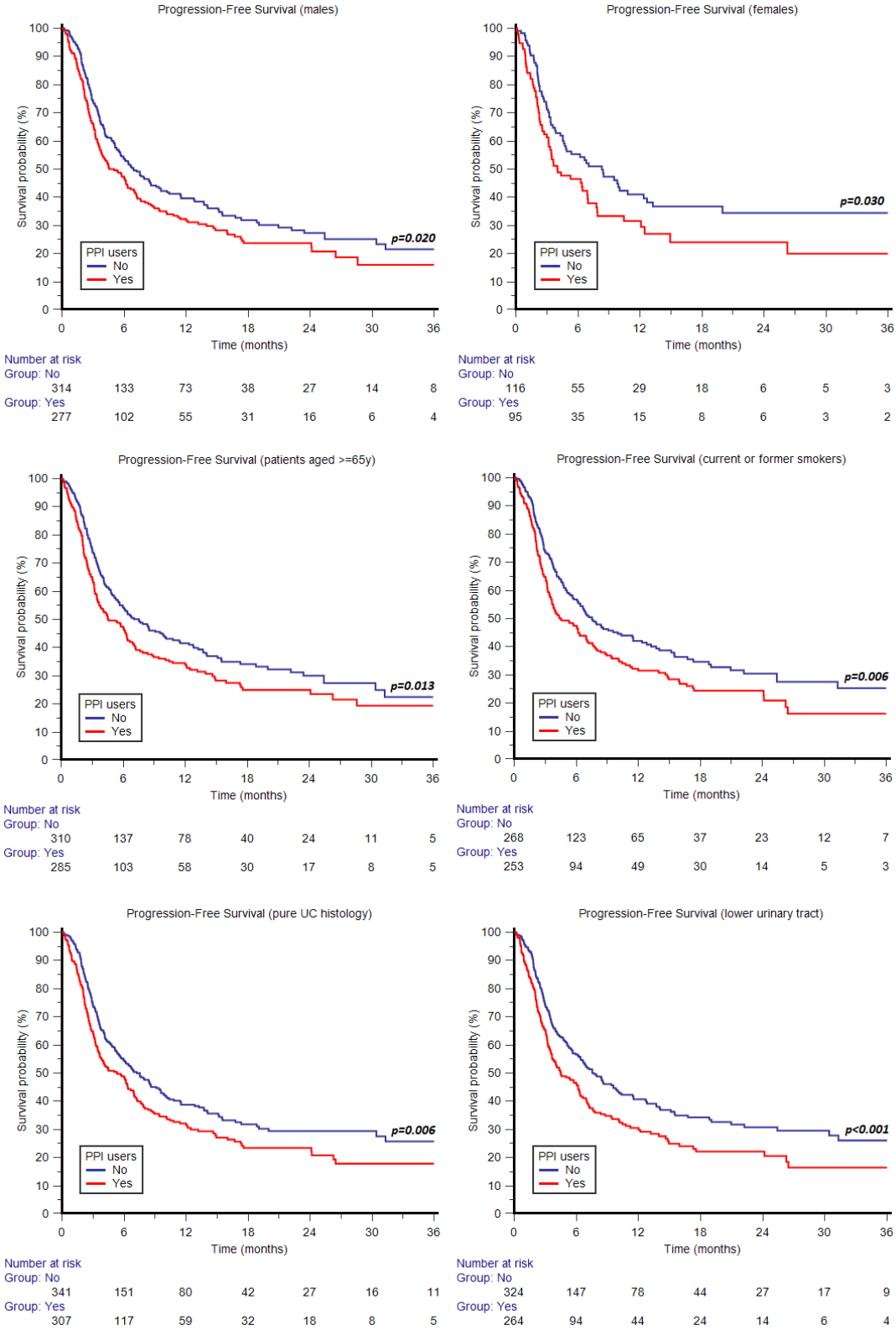
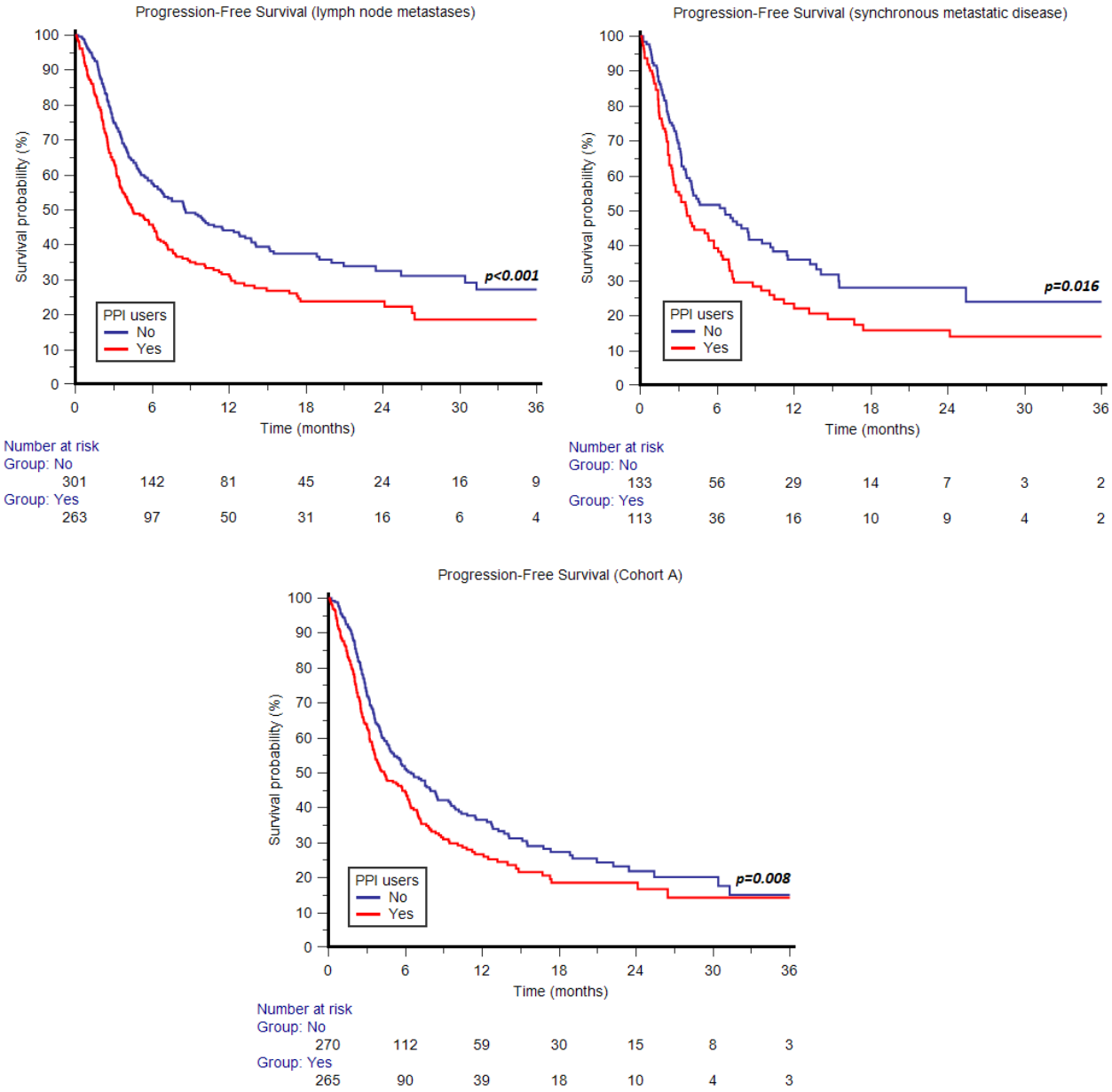


Figure 5. Progression-free survival according to concomitant use of PPIs stratified synchronous metastatic disease, lymph node metastases and pembrolizumab setting (second-line therapy after progression on first-line platinum-based chemotherapy, Cohort A).



Conflicts of Interest

O. Fiala received honoraria from Roche, Janssen, GSK and Pfizer for consultations and lectures unrelated to this project. S. Buti received honoraria as speaker at scientific events and advisory role by BMS, Pfizer, MSD, Ipsen, AstraZeneca, Merck, all unrelated to this project. M. Santoni has received research support and honoraria from Janssen, Bristol Myers Squibb, Ipsen, MSD,Astellas and Bayer, all unrelated to this project. R. Kanesvaran has received fees for speaker bureau and advisory board activities from the following companies; Pfizer, MSD, BMS, Eisai, Ipsen, Johnson and Johnson, Merck, Amgen, Astellas and Bayer, all unrelated to this project. E. Grande has received honoraria for speaker engagements, advisory roles or funding of continuous medical education from Adacap, AMGEN, Angelini, Astellas, Astra Zeneca, Bayer, Blueprint, Bristol Myers Squibb, Caris Life Sciences, Celgene, Clovis-Oncology, Eisai, Eusa Pharma, Genetracer, Guardant Health, HRA-Pharma, IPSEN, ITM-Radiopharma, Janssen, Lexicon, Lilly, Merck KGaA, MSD, Nanostring Technologies, Natera, Novartis, ONCODNA (Biosequence), Palex, Pharmamar, Pierre Fabre, Pfizer, Roche, Sanofi-Genzyme, Servier, Taiho, and Thermo Fisher Scientific and has received research grants from Pfizer, Astra Zeneca, Astellas, and Lexicon Pharmaceuticals, all unrelated to this project. F. S. M. Monteiro has received research support from Janssen, Merck Sharp Dome and honoraria from Janssen, Ipsen, Bristol Myers Squibb and Merck Sharp Dome, all unrelated to this project. C. Porta has received honoraria from Angelini Pharma, Astra Zeneca, BMS, Eisai, General Electric, Ipsen and MSD and acted as a Protocol Steering Committee Member for BMS, Eisai and MSD, all unrelated to this project. Z. Myint has received research support from Merck unrelated to this project. J. Molina-Cerrillo declares consultant, advisory or speaker roles for IPSEN, Roche, Pfizer, Sanofi, Janssen, and BMS and has received research grants from Pfizer, IPSEN and Roche, all unrelated to

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Author Contributions

Manuscript: Use of concomitant proton pump inhibitors, statins, or metformin in patients treated with pembrolizumab for advanced urothelial carcinoma: Data from the ARON-2 retrospective study. Fiala et al.

O. Fiala: Investigation, Writing - Original Draft

M. Santoni: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft

S. Buti: Conceptualization, Methodology, Investigation, Writing - Original Draft

All authors: Investigation