Association of prior local therapy and outcomes with PD(L)1 inhibitors in advanced urothelial cancer

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Abstract:

Objectives: To compare clinical outcomes with anti-PD(L)1 immune checkpoint inhibitors (ICIs) in patients with advanced urothelial carcinoma (aUC) who have vs have not undergone radical surgery (RS) or radiation (RT) prior to developing metastatic disease.

Patients and Methods: We performed a retrospective cohort study collecting clinicopathological, treatment and outcomes data for patients with aUC receiving ICIs across 25 institutions. We compared outcomes (observed response rate [ORR], progression-free survival [PFS], overall survival [OS]) between patients with vs without prior RS, and by type of prior locoregional treatment (RS vs RT vs no locoregional treatment). Patients with *de novo* advanced disease were excluded. Analysis was stratified by treatment line (first [1st] & second or greater [2nd+]). Logistic regression was used to compare ORR; Kaplan-Meier analysis and Cox regression for PFS and OS. Multivariable models were adjusted for known prognostic factors.

Results: We included 562 patients (1st line: 342 and 2nd+: 220). There was no difference in outcomes based on prior locoregional treatment among those treated with 1st line ICI. In the 2nd+ line, prior RS was associated with higher ORR (adjusted odds ratio [aOR] 2.61 [95% CI 1.19-5.74]), longer OS (adjusted hazard ratio [aHR] 0.61 [95% CI 0.42-0.88]) and PFS (aHR 0.63 [95% CI 0.45-0.89]) vs no

prior RS. This association remained significant when the type of prior locoregional treatment (RS and RT) was modeled separately.

Conclusion: Prior RS prior to developing advanced disease was associated with better outcomes in patients with aUC treated with ICI in the 2nd+, but not in the 1st line setting. While further validation is needed, our findings can have implications on prognostic estimates in clinical discussions and benchmarking for clinical trials. Limitations include retrospective nature, lack of randomization, possible selection and confounding biases.

Keywords (3-6): Bladder Cancer, Urinary Tract Neoplasms, Urothelial Carcinoma, Immune Checkpoint Inhibitors, Immunotherapy, Outcomes

Abbreviations:

- aUC: advanced Urothelial Carcinoma
- RT: radiation therapy
- mOS: median Overall Survival
- PFS: Progression-free Survival
- ORR: Observed Response Rate
- HR: Hazard Ratio
- aHR: adjusted HR
- NMIBC: Non-muscle Invasive Bladder Cancer
- ICI: Immune Checkpoint Inhibitors
- UTUC: Upper Tract Urothelial Carcinoma
- LTUC: Lower Tract Urothelial Carcinoma
- CR: Complete Response
- PR: Partial Response
- SD: Stable Disease
- PD: Progressive Disease

INTRODUCTION:

Bladder cancer is one of the most common malignancies worldwide and the 6th most prevalent in the United States [1]. Locoregional treatment with radical surgery (RS; cystectomy or (nephro)ureterectomy) with lymph node dissection (LND) (ideally with neoadjuvant cisplatin-based

chemotherapy) is the most accepted treatment for non-metastatic muscle invasive urothelial cancer [2]. However, over half of patients undergoing RS develop recurrence with poor outcomes [3-5].

Over the last five years, immune checkpoint inhibitors (ICIs) have become an established treatment option for patients with locally advanced, unresectable or metastatic urothelial carcinoma (aUC). These agents improve overall survival (OS) when given in the 2^{nd+} line (post-platinum progression, based on Keynote 045 trial) or switch maintenance setting (based on Javelin Bladder 100 trial) [6-8]. There are also FDA accelerated approvals for pembrolizumab and atezolizumab in the 1^{st} line setting for cisplatin-ineligible patients with PD-L1 high (CPS≥10 and IC2/3, respectively) tumors and for patients who are platinum (cisplatin and carboplatin) ineligible based on single arm phase II trials [8]. While ICIs have significantly improved outcomes in patients with aUC, response rates and progression-free survival (PFS) remain modest, and cure remains elusive while there is a notable risk of immune related adverse events. Therefore, clinical and molecular biomarkers are needed to help identify patients who are more likely to benefit from ICIs.

In advanced kidney cancer, retrospective studies suggest overall survival with ICI-based therapy was longer in patients with prior nephrectomy compared to those without[9] but prospective trials are further investigating this question (PROBE trial; NCT04510597). However, the potential impact of the presence of the primary tumor on ICI response and outcomes in aUC remains unclear. It could be hypothesized that the primary tumor may either potentially serve as a “neoantigen beacon”, providing a plethora of neoantigens bolstering anti-tumor immune response, or, on the contrary, may exert immunosuppressive effects dampening response to ICI. To address this question, we conducted a retrospective cohort study investigating the potential association between prior definitive locoregional treatment, in the form of RS or RT, response and survival with subsequent ICIs given for aUC. We hypothesized that prior RS would be associated with higher response rates and longer survival to subsequent ICIs given for aUC.

PATIENTS AND METHODS:

Study design, patients and data collection:

After institutional review board approval was attained in concordance with the Declaration of Helsinki, we performed a retrospective cohort study, using a cohort [10-13] of patients from 25 institutions in the United States and Europe. Consecutive patients at each institution were identified using a combination of provider-driven and electronic health record search algorithms. Patients were included if they had been treated with ICI monotherapy for aUC with prior locoregional only disease. All patients with RS included had RS prior to development of metastatic disease. Patients were excluded if they had

missing data, *de novo* advanced disease (unresectable or metastatic), had received multiple lines of ICIs, if ICI was given for a different indication, on a trial or in combination with other systemic therapy, if patients had pure non-UC histology, if RS history was unknown, or if they underwent palliative RS in the advanced disease setting (Figure 1).

For data collection and storage, we used web-based, secure and standardized REDCap capture tools hosted at the Institute of Translational Sciences [14,15]. Data collected included demographics, histology type, whether prior RS or RT was performed, laboratory values, sites of metastatic disease and outcomes (response, progression, death). Pathology and radiology results were assessed based on notes in the electronic health record; no central review of either was performed. All patients underwent imaging at the discretion of treating provider. Evaluation of response and progression were determined by the chart abstractor based on best available information in notes and radiographic studies.

2.Statistical analysis:

Baseline characteristics were summarized using descriptive statistics and compared via chi-square and Student's t-tests, for categorical and continuous variables, respectively. Observed response rate (ORR) was determined on an investigator-provider basis and calculated as the sum of patients with response divided by the total number of patients. OS was defined as time from ICI initiation to date of death, and PFS as time from ICI initiation until date of radiographic or clinical progression or death. Patients who did not experience death or progression were censored at the date of last follow-up.

All outcomes (ORR, PFS, OS) were analyzed separately for patients treated with ICI in the first line (1^{st}) and the subsequent setting (second line and beyond; 2^{nd+}). For the primary analysis, we compared those with prior RS to those without prior RS. We also did a secondary analysis comparing those with prior RS vs those with prior RT vs those with neither. The very few patients who had received both RS and RT were classified as recipients of RS for this secondary analysis. We also did an exploratory analysis comparing patients with lower tract (LT)UC and those with upper tract (UT)UC. We assume that those with LTUC had cystectomy and those with UTUC had (nephro)ureterectomy, though the specific type of RS was not explicitly collected in the survey tool. In both analyses, the group without history of locoregional therapy served as the reference group for all comparisons. We used univariable and multivariable logistic regression to estimate the odds ratios (OR) and confidence intervals (CI) for ORR. For OS and PFS, we estimated medians using the Kaplan-Meier method and compared groups using univariable and multivariable Cox Regression. For the multivariable analysis, models were adjusted based on calculated risk scores developed to prognosticate shorter OS; an internally developed risk score (ECOG PS ≥2, neutrophil:lymphocyte ratio>5, albumin <3.5.g/dL, liver metastases [11]) was used for 1^{st} line and Bellmunt [16] for 2^{nd+} line analysis. The alpha value was set

at 0.05 for all analyses. Analyses were performed with Stata IC 16.0 (Stata LLC, College Station, Texas)

RESULTS

Patient Characteristics

Data from 1,026 patients with aUC treated with ICI across 25 institutions were available with 537, 537 and 554 ultimately included in the ORR, OS and PFS analyses, respectively (Figure 1). Table 1 shows the baseline characteristics for patients with and without prior RS, stratified by line of therapy (1st and 2nd+). Among 342 and 220 patients treated with 1st and 2nd+ line ICIs, 230 (67%) and 144 (65%) had prior RS, respectively. Median time interval from RS to recurrence was 255 days (interquartile range [IQR] 101-479) in the 1st and 422 (IQR 187-852) in the 2nd+ line subgroups, respectively. Upper tract urothelial cancer accounted for 14% in the 1st and 17% in the 2nd+ line with a significantly higher proportion of patients with UTUC having had prior RS in both treatment setting subgroups. Among those without prior RS, 38 had received locoregional RT; 24 were treated with ICI in the 1st line setting and 14 in the 2nd+ line setting. Only five patients had undergone both prior RS and RT.

In the 1st line setting, patients with prior RS were slightly younger (median 70 vs 74 years), with a significantly higher prevalence of white race, UTUC, presence of liver metastases, and prior receipt of platinum-based chemotherapy in the neoadjuvant or adjuvant setting. For those treated with ICIs in 2nd+ line setting, a significantly greater proportion with prior RS had UTUC and liver metastases and fewer patients had albumin<3.5g/dL and hemoglobin<10g/dL at the time of ICI initiation. Otherwise, in both the 1st and 2nd+ line subgroups, the distribution of risk scores (internally developed/published and Bellmunt [12,17]) was not significantly different between those with and without prior RS.

2. Observed response rate (ORR)

A total of 537 patients were included in ORR analysis; 324 and 213 patients were treated with ICI in the 1st and 2nd+ line setting, respectively. ORR between groups in the 1st line was not significantly different – those with prior RS had ORR of 28% (95% CI 23-34) and those without had ORR of 33% (95% CI 24-42; Table 2). However, among those treated with ICI in 2nd+,line, ORR was 32% (95% CI 25-40) and 15% (95% CI 9-25) for those with and without prior RS, respectively (adjusted odds ratio [aOR] 2.61 [95% CI 1.19-5.74]; Table 2). When type of locoregional therapy was considered in the model (no locoregional therapy vs RS vs RT), ORR with ICIs remained significantly higher for those with prior RS in 2nd+ line, but not in 1st (Table 3). Upon analyzing data based on the site of primary tumor, ORR was similar in the 1st line, but in the 2nd+ line, patients with prior RS for UTUC

demonstrated lower ORR compared to those with LTUC (20% vs 36%; aOR 0.31 (95% CI 0.10-0.98 [Table S1]).

3. Progression-free survival

A total of 554 patients were included in PFS analysis; 339 and 215 were treated with ICI in the 1st and 2nd+ line respectively. Median PFS for those with and without prior RS was 4 (95% CI 3-5) and 6 months (95% CI 3-7) in 1st, and 5 (95% CI 4-7) and 3 months (95% CI 2-4) in 2nd+ line, respectively. Prior RS was associated with longer PFS in the 2nd+, but not the 1st line setting (adjusted hazard ratio [aHR] 0.63 [95% CI 0.45-0.89]; Table 2; Figure 3). This association of longer PFS in the 2nd+ line with prior RS was also observed in the 3-factor locoregional therapy model (Table 3; Figure S1). In the exploratory analysis based on the site of the primary tumor, no significant difference in PFS was noted (Table S1).

4. Overall survival

A total of 537 patients were included in the OS analysis; 330 with 1st line ICI and 207 with 2nd+ line ICI. In the 1st line subgroup, OS between patients with vs without prior RS was similar (10 [95% CI 7-13] vs 11 months [95% CI 7-14]; aHR 1.10; **Table 2**, **Figure 2**). In the 2nd+ line setting, patients with prior RS had longer median OS (11 [95% CI 8-18] vs 5 months [95% CI 4-10]), which was a significant association in our multivariable analyses (HR 0.61 [95% CI 0.42-0.88]; **Table 2, Figure 2**). Upon comparing patients based on the 3-factor locoregional therapy model, prior RS remained significantly associated with longer OS in the 2nd+ line (**Table 3, Figure S2**). In the exploratory analysis based on the site of the primary tumor, no significant difference in OS was noted (Table S1).

DISCUSSION:

In this retrospective cohort study of patients with aUC treated with ICIs, history of prior RS was associated with higher ORR, as well as longer OS and PFS with ICI in the 2nd+, but not the 1st line, setting. Prior definitive RT was associated with numerically, but not statistically significant, higher ORR, longer OS and PFS, a finding that might be attributed to the small sample size for this particular subset.

The association between prior RS, ORR and OS with ICIs has not been well studied in aUC. To our knowledge, clinical trials of ICIs in aUC have not definitely investigated the association between prior RS and outcomes with ICI. Prior retrospective studies from other solid tumor malignancies have suggested that history of resection of the primary tumor can be associated with better outcomes with ICI therapy. Amin et al. investigated outcomes with ICIs among patients with non-small lung cancer, breast cancer, melanoma, colorectal cancer or kidney cancer who had brain metastases and noted

longer OS among those with history of surgical resection of the primary tumor [17]. Similarly, Singla et al. compared outcomes among patients with RCC treated with immunotherapy alone or with cytoreductive nephrectomy and noted longer OS for those treated with cytoreductive nephrectomy and immunotherapy [9]. Selection and confounding biases may impact the outcome of such retrospective studies, with performance status, frailty and overall fitness being major potential confounders. Prospective clinical trials may ultimately help answer similar questions (e.g. S1931/PROBE).

In the setting of aUC, surgical considerations such as RS remain controversial. Radical cystectomy is associated with morbidity and has not been shown to definitively improve outcomes. In a molecular level, metastatic disease may have tumor heterogeneity, rendering the radical treatment of only part of the disease less valuable. Establishment of metastases implies the presence of circulating tumor cells seeding distant tissues, making surgery seem futile.

Despite such concerns, prior retrospective studies in patients with aUC treated with cytoreductive or palliative cystectomy have also suggested that this approach might be appropriate for a sub-population of patients with aUC. Li et al. [18] found that post-chemotherapy RS was associated with longer OS in patients with aUC compared with those treated with local radiation or no local therapy. A review by Abufaraj et al. [19] suggested that RS may be beneficial for patients with prior response to chemotherapy and low volume of disease. Moschini et al. [20] also reported a significant survival benefit for patients with up to one metastatic site. These results, despite their several inherent caveats, raise the question whether carefully-selected patients with aUC, especially those with very well controlled disease and indolent course, might potentially benefit from RS. However, it remains unclear whether there is any interaction between RS (and its timing over the disease course) with response and outcomes with ICIs. While most of those studies in aUC or other solid tumors investigate the role of extirpative surgery in the advanced setting, our results suggest that prior RS history for locoregional only disease may also portend favorable prognosis.

It was also notable that in our analysis of RS vs no-RS, non-RS treated patients included those with history of prior RT, which may have acted as a confounding factor. In the 3-factor model for analysis of the prior locoregional therapy type, separation of RT-treated patients from those without history of any locoregional therapy showed that the latter subset had significantly worse outcomes compared to those treated with prior RS.

Locoregional RT has been associated with greater response to ICI and a possible role for abscopal effect has been suggested [21, 22]. However, when the abscopal effect was noted in studies, radiotherapy was administered concurrently to ICI, whereas in our study RT was given prior to

development of metastatic disease and ICIs were given for metastatic disease, so RT and ICI were not given together and for most patients the time between RT and ICI was extensive. The relative timing of RT and ICI administration was recently tested in a randomized phase I trial comparing pembrolizumab with sequential vs concomitant stereotactic body RT (SBRT) to a single metastatic lesion in 18 patients with aUC [23]. In this study, none of the nine patients randomized to sequential therapy had response (ORR 0%), whereas the ORR for those receiving concurrent RT was 44%. While the small sample and selection bias of this study require further external validation, those results suggest potential synergy between concomitant RT and ICIs, but not sequential therapy. Our cohort did not demonstrate a clear signal of abscopal effect; patients had received RT before initiation of ICI, not concurrently, with variable time intervals between the two. However, our study showed that prior locoregional RT had a trend towards better outcomes, but did not reach statistical significance, which could suggest a possible association that was limited by the small sample size and warrants further evaluation in larger cohorts.

In our cohort, response rates in the 2^{nd+} line setting were significantly greater among patients treated with prior locoregional treatment (ORR; 32% and 36% for RS and RT recipients, respectively, vs only 10% for those without prior locoregional therapy). This substantial difference could possibly be influenced by confounding factors, such as older age, worse ECOG PS or medical comorbidities that can render patients poor RT/RS candidates and also dampen immune response with ICI. It is notable that in an exploratory analysis of cisplatin-ineligible patients of more senior age and poor PS in the KN-052 study, ORR was not significantly different in any subgroup, including among patients ≥65 and ≥75 years with ECOG PS 2 [24]. Prior work from our registry has also shown that patients with ECOG PS ≥2 had similar ORR to those with ECOG PS 0-1, though none of the 11 patients with ECOG PS 3 had response [13]. In our current analysis, we considered the possible confounding influence by including in our multivariable model risk scores (internally developed risk factor model [11] for 1st line [adjusting for ECOG PS ≥ 2, neutrophil:lymphocyte ratio>5, albumin <3.5 g/dL, liver metastasis] and for Bellmunt risk factors [16] for 2^{nd+}) that have been developed to help discriminate patients with different outcomes. Therefore, the notion of potential positive impact of prior RS to ICI response remains plausible.

While history of RS was associated with a higher response rate to ICI and longer survival in the 2^{nd+} line, this association was not significant in the 1st line. This discordance may possibly be attributed to confounding factors. Patients treated with ICI in the 1st line setting can often be cisplatin-ineligible due to frailty and/or comorbidities, such as chronic kidney disease. Moreover, PDL1 expression can be used for patient selection for ICI in the 1st line setting of cisplatin-ineligible patients, which can impact

response and survival in this setting, while PDL1 is not used for patient selection in the 2nd+ line. This and other potential confounding factors may determine therapy response and outcomes in addition to specific anti-cancer therapies. In the 2^{nd+} line, patients without prior RS may be substantially more frail / less fit, which can further impact the outcome in this pretreated clinical setting. The overall state of health of individuals receiving ICI can possibly be associated with the robustness of the immune system, a factor likely also affecting immune system response.

Our analysis comparing outcomes with ICI based on the primary tumor site (UTUC vs LTUC) showed that patients with prior RS with LTUC had significantly higher ORR in the 2^{nd+} line setting compared to those with UTUC. Our team previously investigated the association of primary tumor site with response to ICI, demonstrating no significant differences [12] between UTUC and LTUC in all evaluated patients. It is possible that the difference detected in our present analysis might be attributed to the relatively small sample size as the 2^{nd+} line group consisted of 109 patients with LTUC and 30 with UTUC, as well as selection of patients with prior RS.

Our study is retrospective and thus can be interpreted with caution. Our results provide insights on the association between prior RS and response to ICI, raising the hypothesis that RS may possibly be associated with future ICI response after subsequent development of advanced disease. Our results cannot guarantee that differences in ICI response and survival are solely due to RS, and, not other confounding factors, such as patient performance status and comorbidities. However, one may extrapolate that RS may be considered in borderline resectable cases in the absence of metastatic disease and, therefore, can influence such informed/shared decision making. Moreover, the decision for administering ICI in aUC should not be based on whether the patient had prior RS or not, but our data may inform clinical discussions about prognostication and risk stratification.

Our results may also inform interpretation of clinical trials evaluating ICIs in aUC. For example, the proportion of patients with prior RS in clinical trials may impact response and survival rates with ICIs. The selection of an ICI (pembrolizumab) vs Fibroblast Growth Factor Receptor (FGFR) inhibitor, erdafitinib, in the platinum-refractory setting is being assessed in the phase III THOR trial; the proportion of patients with prior RS enrolled may possibly affect the endpoint. Another potential indirect implication is the discussion about the optimal design and duration of ICI therapy in perioperative clinical trials. For example, multiple phase II clinical trials with ICIs in the neoadjuvant setting have shown promising efficacy, measured by pathologic complete response rate [25-28]. However, the question remains whether ICI should be tested in the neoadjuvant or adjuvant, or both settings. Primary analysis from the adjuvant IMvigor 010 trial did not detect a significant disease-free survival benefit with atezolizumab vs observation [29], while the Checkmate 274 trial [30] showed

significantly prolonged disease-free survival with adjuvant nivolumab vs placebo. Since the presence of the primary tumor may possibly compromise ORR and survival with ICIs in aUC, a generated hypothesis is that the adjuvant component may be relevant in peri-operative trials. However, the different disease settings (localized vs metastatic) can significantly limit the extrapolation of our findings to the perioperative treatment scenario.

Our study has several limitations inherent to the retrospective study design, including lack of randomization, potential selection bias and residual confounding. In addition, clinical practices, surveillance schedules and follow up times may vary, while documentation might not be perfectly consistent across all 25 institutions. We did not have centralized review of pathology or imaging, but all participating sites are reference academic sites with expert genitourinary oncologists, radiologists and pathologists. Detailed clinical staging information prior to locoregional therapy was not available, as well as data on response to perioperative chemotherapy. Radical surgery approaches may also vary between institutions; however, all institutions were academic centers with dedicated genitourinary oncologists with relevant expertise, so there is likely no significant variability in the quality of RS. Response and progression were determined by systematic comprehensive chart review based on clinical and radiological notes without mandating formal, pre-specified interval assessments via RECIST 1.1 criteria. However, our study also had strengths including the utilization of 'real-world' data, participation of multiple institutions on two continents, and a relatively large sample size.

In conclusion, our study demonstrated a significantly higher ORR, longer OS and PFS with ICI in the 2^{nd+} line setting for patients with aUC and prior RS compared to those with no prior RS. Despite inherent limitations, this analysis provides insight into an important topic that has not been extensively studied. Future research is needed to identify biomarkers and clinical tools that can better identify patients with aUC more likely to benefit from ICIs.

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REFERENCES:

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018 Jan;68(1):7-30. doi: 10.3322/caac.21442. Epub 2018 Jan 4. PMID: 29313949.

2. Abufaraj M, Gust K, Moschini M, *et al*. Management of muscle invasive, locally advanced and metastatic urothelial carcinoma of the bladder: a literature review with emphasis on the role of

surgery. *Transl Androl Urol*. 2016;5(5):735-744. doi:10.21037/tau.2016.08.23.

3. M. Moschini, S. Zamboni, J.R. Karnes *et a*l 845 - Clinical recurrence after radical cystectomy for bladder cancer, defining optimal surveillance after surgery, European Urology Supplements, Volume 18, Issue 1, 2019,
4. Mari, A., Campi, R., Tellini, R. *et al.* Patterns and predictors of recurrence after open radical cystectomy for bladder cancer: a comprehensive review of the literature. *World J Urol* 36, 157–170 (2018). https://doi.org/10.1007/s00345-017-2115-4
5. Paul Sargos, Brian C. Baumann, Libni Eapen, *et a*l Risk factors for loco-regional recurrence after radical cystectomy of muscle-invasive bladder cancer: A systematic-review and framework for adjuvant radiotherapy, Cancer Treatment Reviews, Volume 70,2018, Pages 88-97, ISSN 0305-7372
6. Powles T, Park SH, Voog E, et al. Avelumab Maintenance Therapy for Advanced or Metastatic Urothelial Carcinoma. *N Engl J Med*. 2020;383(13):1218-1230. doi:10.1056/NEJMoa2002788
7. Powles T, Durán I, van der Heijden MS, *et al*. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial *Lancet*. 2018;391(10122):748-757. doi:10.1016/S0140-6736(17)33297-X
8. Gopalakrishnan D, Koshkin VS, Ornstein MC, *et al*. Immune checkpoint inhibitors in urothelial cancer: recent updates and future outlook. Ther Clin Risk Manag. 2018 Jun 5;14:1019-1040. doi: 10.2147/TCRM.S158753. PMID: 29892196; PMCID: PMC5993034
9. Nirmish Singla, Ryan C. Hutchinson, Rashed A. Ghandour, *et al* Improved survival after cytoreductive nephrectomy for metastatic renal cell carcinoma in the contemporary immunotherapy era: An analysis of the National Cancer Database,Urologic Oncology: Seminars and Original Investigations, Volume 38, Issue 6,2020, Pages 604.e9-604.e17, ISSN 1078-1439
10. Miller NJ, Khaki AR, Diamantopoulos LN, *et al.* Histological Subtypes and Response to PD-1/PD-L1 Blockade in Advanced Urothelial Cancer: A Retrospective Study. *J Urol*. 2020;204(1):63-70. doi:10.1097/JU.0000000000000761
11. Khaki AR, Li A, Diamantopoulos LN, *et al*. A New Prognostic Model in Patients with Advanced

Urothelial Carcinoma Treated with First-line Immune Checkpoint Inhibitors [published online ahead of print, 2021 Jan 7]. *Eur Urol Oncol*. 2021;S2588-9311(20)30214-5. doi:10.1016/j.euo.2020.12.006

12. Esagian, S.M., Khaki, A.R., Diamantopoulos, L.N.*et al*. (2021), Immune checkpoint inhibitors in advanced upper and lower tract urothelial carcinoma: a comparison of outcomes. BJU Int. https://doi.org/10.1111/bju.15324

13. Khaki, A.R., Li, A., Diamantopoulos, L.N., *et al* (2020), Impact of performance status on treatment outcomes: A real-world study of advanced urothelial cancer treated with immune checkpoint inhibitors. Cancer, 126: 1208-1216. https://doi.org/10.1002/cncr.32645

14. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208. doi:10.1016/j.jbi.2019.103208

15. Harris PA, Taylor R, Thielke R, *et al* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009 Apr;42(2):377-81. doi: 10.1016/j.jbi.2008.08.010. Epub 2008 Sep 30. PMID: 18929686; PMCID: PMC2700030.

16. Bellmunt J, Choueiri TK, Fougeray R, et al. Prognostic factors in patients with advanced transitional cell carcinoma of the urothelial tract experiencing treatment failure with platinum-containing regimens. *J Clin Oncol*. 2010;28(11):1850-1855. doi:10.1200/JCO.2009.25.4599

17. Amin S, Baine MJ, Meza JL, Lin C. Association of Immunotherapy With Survival Among Patients With Brain Metastases Whose Cancer Was Managed With Definitive Surgery of the Primary Tumor. *JAMA Netw Open.* 2020;3(9):e2015444. doi:10.1001/jamanetworkopen.2020.15444

18. Li R, Metcalfe M, Kukreja J, Navai N. Role of Radical Cystectomy in Non-Organ Confined Bladder Cancer: A Systematic Review. *Bladder Cancer*. 2018;4(1):31-40. Published 2018 Jan 20. doi:10.3233/BLC-170130

19. Abufaraj M, Dalbagni G, Daneshmand S, et al. The Role of Surgery in Metastatic Bladder Cancer: A Systematic Review. *Eur Urol*. 2018;73(4):543-557. doi:10.1016/j.eururo.2017.09.030

20. Moschini M, Xylinas E, Zamboni S, et al. Efficacy of Surgery in the Primary Tumor Site for Metastatic Urothelial Cancer: Analysis of an International, Multicenter, Multidisciplinary Database. *Eur Urol Oncol*. 2020;3(1):94-101. doi:10.1016/j.euo.2019.06.014

21. Rompré-Brodeur A, Shinde-Jadhav S, Ayoub M, et al. PD-1/PD-L1 Immune Checkpoint Inhibition with Radiation in Bladder Cancer: *In Situ* and Abscopal Effects. *Mol Cancer Ther*. 2020;19(1):211-220. doi:10.1158/1535-7163.MCT-18-0986

22. Daro-Faye M, Kassouf W, Souhami L, et al. Combined radiotherapy and immunotherapy in urothelial bladder cancer: harnessing the full potential of the anti-tumor immune response [published online ahead of print, 2020 Sep 11]. *World J Urol*. 2020;1-13. doi:10.1007/s00345-020-03440-4

23. Sundahl N, Vandekerkhove G, Decaestecker K, et al. Randomized Phase 1 Trial of Pembrolizumab with Sequential Versus Concomitant Stereotactic Body Radiotherapy in Metastatic Urothelial Carcinoma. *Eur Urol*. 2019;75(5):707-711. doi:10.1016/j.eururo.2019.01.009

24. Grivas P, Plimack ER, Balar AV, et al. Pembrolizumab as First-line Therapy in Cisplatin-ineligible Advanced Urothelial Cancer (KEYNOTE-052): Outcomes in Older Patients by Age and Performance Status. *Eur Urol Oncol*. 2020;3(3):351-359. doi:10.1016/j.euo.2020.02.009

25. Horowitz M, Neeman E, Sharon E, Ben-Eliyahu S. Exploiting the critical perioperative period to improve long-term cancer outcomes. *Nat Rev Clin Oncol*. 2015;12(4):213-226. doi:10.1038/nrclinonc.2014.224

26. Necchi A, Anichini A, Raggi D, *et al*. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-Label, Single-Arm, Phase II Study. *J Clin Oncol*. 2018;36(34):3353-3360. doi:10.1200/JCO.18.01148

27. Powles, T., Kockx, M., Rodriguez-Vida, A. *et al.* Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. *Nat Med* **25,**

1706–1714 (2019). https://doi.org/10.1038/s41591-019-0628-7

28. van Dijk, N., Gil-Jimenez, A., Silina, K. *et al.* Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: the NABUCCO trial. *Nat Med* **26,** 1839–1844 (2020). https://doi.org/10.1038/s41591-020-1085-z

29. Maha H. A. Hussain, Thomas Powles, Peter Albers, *et al* IMvigor010: Primary analysis from a phase III randomized study of adjuvant atezolizumab (atezo) versus observation (obs) in high-risk muscle-invasive urothelial carcinoma (MIUC) Journal of Clinical Oncology 2020 38:15_suppl, 5000-5000

30. Bajorin DF, Witjes JA, Gschwend J, Schenker M. First results from the phase 3 CheckMate 274 trial of adjuvant nivolumab vs placebo in patients who underwent radical surgery for high-risk muscle-invasive urothelial carcinoma (MIUC). *J Clin Oncol* 39, 2021 (suppl 6; abstr 391). doi: 10.1200/JCO.2021.39.6_suppl.391

Table 1. Baseline characteristics for patients with advanced urothelial carcinoma treated with checkpoint inhibitors, stratified by treatment line and prior radical surgery

	First line ICI			Second or later line ICI		
Radical Surgery	**No**	**Yes**	**P**	**No**	**Yes**	**P**
Number of Patients	112	230		76	144	
Age at ICI initiation, median (IQR)	74 (67-82)	70 (61-77)	<0.001	71 (61-77)	70 (64-74)	0.48
Male, N (%)	83 (74)	168 (73)	0.83	64 (84)	111 (77)	0.21
Ever Smoker, N (%)	78 (70)	159 (69)	0.99	58 (76)	103 (71)	0.45
White race, N (%)	74 (66)	179 (78)	0.02	52 (68)	115 (80)	0.06
Pure UC, N (%)	81 (72)	153 (67)	0.30	53 (70)	111 (77)	0.20
Upper tract UC, N (%)	10 (9)	39 (17)	0.05	7 (9)	30 (21)	0.03
Prior platinum chemotherapy, N (%)	25 (22)[a]	149 (65)	<0.001	76 (94)	131 (94)	0.63
Days from RS to metastatic progression, median (IQR)	NA	255 (101-479)	NA	NA	422 (187-852)	NA
Albumin<3.5 g/dL at ICI initiation, N (%)	30 (27)	57 (25)	0.77	26 (34)	23 (16)	0.002
Hgb<10 g/dL at ICI initiation, N (%)	33 (30)	49 (21)	0.11	26 (34)	31 (22)	0.04
Liver Metastasis at ICI initiation, N (%)	12 (11)	45 (20)	0.04	10 (13)	38 (26)	0.02
ECOG Performance Status, N (%)						
0	20 (18)	50 (22)	0.41	7 (9)	24 (17)	0.03

1	60 (54)	101 (44)		47 (58)	85 (61)	
2	23 (21)	47 (20)		14 (17)	19 (14)	
3	5 (5)	4 (2)		5 (6)	1 (1)	
4	0 (0)	1 (<1)		0 (0)	0 (0)	
Missing	4 (4)	27 (12)		8 (10)	11 (8)	
Risk Score[b]						
0	40 (36)	66 (29)	0.44	6 (7)	18 (13)	0.31
1	29 (26)	61 (27)		37 (46)	66 (47)	
2	18 (16)	34 (15)		26 (32)	34 (24)	
3[c]	13 (12)	24 (10)		3 (4)	10 (7)	
Missing	12 (11)	45 (20)		9 (11)	12 (9)	
ICI Received						
Atezolizumab	39 (35)	83 (36)	0.74	46 (57)	74 (53)	0.55
Avelumab	0 (0)	1 (<1)		2 (3)	1 (1)	
Durvalumab	5 (5)	5 (2)		1 (1)	3 (2)	
Nivolumab	5 (5)	12 (5)		5 (6)	16 (11)	
Pembrolizumab	62 (55)	125 (54)		26 (32)	45 (32)	

[a] Six patients received platinum-based chemotherapy with radiation; 19 received neoadjuvant platinum chemotherapy but did not proceed to cystectomy (either due to progression or patient preference)

[b] First line: Internally developed risk score [11]; Second or later line: Bellmunt risk score [16]

[c] First-line risk score includes four factors thus score of 3 is ≥3

Table 2. Observed response rate (ORR), Progression-Free Survival and Overall Survival according to prior radical surgery status, stratified by treatment line

Observed Response Rate				
Treatment Line	**History of Radical Surgery?**	**ORR (%) (95% CI)**	**Univariable, OR (95% CI)**	**Multivariable[a] OR (95% CI)**
1st Line	No (n = 107)	33 (24-42)	Reference	Reference
	Yes (n = 217)	28 (23-34)	0.80 (0.49-1.33)	0.73 (0.42-1.27)
2nd+ Line	No (n = 73)	15 (9-25)	Reference	Reference
	Yes (n = 140)	32 (25-40)	2.67 (1.28-5.57)[b]	2.61 (1.19-5.74)[b]
Progression-Free Survival				
		mPFS, months (95% CI)	**Univariable HR (95% CI)**	**Multivariable[a] HR (95% CI)**

1st Line	No (n = 112)	6 (3-7)	Reference	Reference
	Yes (n = 227)	4 (3-5)	1.12 (0.85-1.48)	1.22 (0.89-1.66)
2nd+ line	No (n = 73)	3 (2-4)	Reference	Reference
	Yes (n = 142)	5 (4-7)	0.63 (0.45-0.87)[b]	0.63 (0.45-0.89)[b]
Overall Survival				
		mOS, months (95% CI)	**Univariable HR (95% CI)**	**Multivariable[#] HR (95% CI)**
1st Line	No (n = 108)	11 (7-14)	Reference	Reference
	Yes (n = 222)	10 (7-13)	1.02 (0.75-1.38)	1.10 (0.79-1.53)
2nd+ line	No (n = 70)	5 (4-10)	Reference	Reference
	Yes (n = 137)	11 (8-18)	0.65 (0.46-0.93)[b]	0.61 (0.42-0.88)[b]

[a]Adjusted for internally developed risk score [11] for first line and Bellmunt score for subsequent line [16]

Abbreviations: ORR: observed response rate; OR: odds ratio; PFS: Progression-Free Survival; HR: Hazard Ratio; CI: Confidence Interval

Table 3. Observed Response Rate (ORR), Progression-Free Survival and Overall survival according to prior definitive locoregional therapy, stratified by treatment line

Observed Response Rate				
Treatment Line	**Definitive locoregional therapy**	**ORR (%) (95% CI)**	**Univariable, OR (95% CI)**	**Multivariable[a] OR (95% CI)**
1st line	No surgery or radiation (n = 83)	33 (23-43)	Reference	Reference
	Radical surgery (n = 217)	28 (23-34)	0.81 (0.47-1.40)	0.77 (0.43-1.40)
	Definitive radiation (n = 24)	33 (18-54)	1.04 (0.39-2.73)	1.25 (0.43-3.63)

	No surgery or radiation (n = 59)	10 (5-21)	Reference	Reference
2nd+ line	Radical surgery (n = 140)	32 (25-40)	4.18 (1.67-10.48)[b]	3.76 (1.46-9.69)[b]
	Definitive radiation (n = 14)	36 (16-63)	4.91 (1.23-19.59)[b]	4.30 (0.92-20.13)
Progression-Free Survival				
		mPFS, months (95% CI)	**Univariable HR (95% CI)**	**Multivariable[a] HR (95% CI)**
	No surgery or radiation (n = 88)	5 (3-9)	Reference	Reference
1st line	Radical surgery (n = 227)	4 (3-5)	1.12 (0.83-1.52)	1.18 (0.84-1.65)
	Definitive radiation (n = 24)	7 (2-13)	1.01 (0.56-1.81)	0.86 (0.46-1.63)
	No surgery or radiation (n = 59)	3 (2-4)	Reference	Reference
2nd+ line	Radical surgery (n = 142)	5 (4-7)	0.60 (0.42-0.86)[b]	0.58 (0.40-0.84)[b]
	Definitive radiation (n = 14)	4 (1-10)	0.83 (0.44-1.57)	0.71 (0.36-1.39)
Overall Survival				
		mOS, months (95% CI)	**Univariable HR (95% CI)**	**Multivariable[a] HR (95% CI)**
	No surgery or radiation (n = 85)	12 (7-19)	Reference	Reference
1st line	Radical surgery (n = 222)	10 (7-13)	1.06 (0.76-1.47)	1.09 (0.76-1.56)
	Definitive radiation (n = 23)	10 (3-14)	1.19 (0.66-2.16)	0.96 (0.50-1.82)
	No surgery or radiation (n = 55)	5 (4-9)	Reference	Reference
2nd+ line	Radical surgery (n = 137)	11 (8-18)	0.62 (0.42-0.91)[b]	0.57 (0.38-0.85)[b]

	Definitive radiation (n = 14)	10 (2-21)	0.93 (0.46-1.86)	0.75 (0.36-1.57)

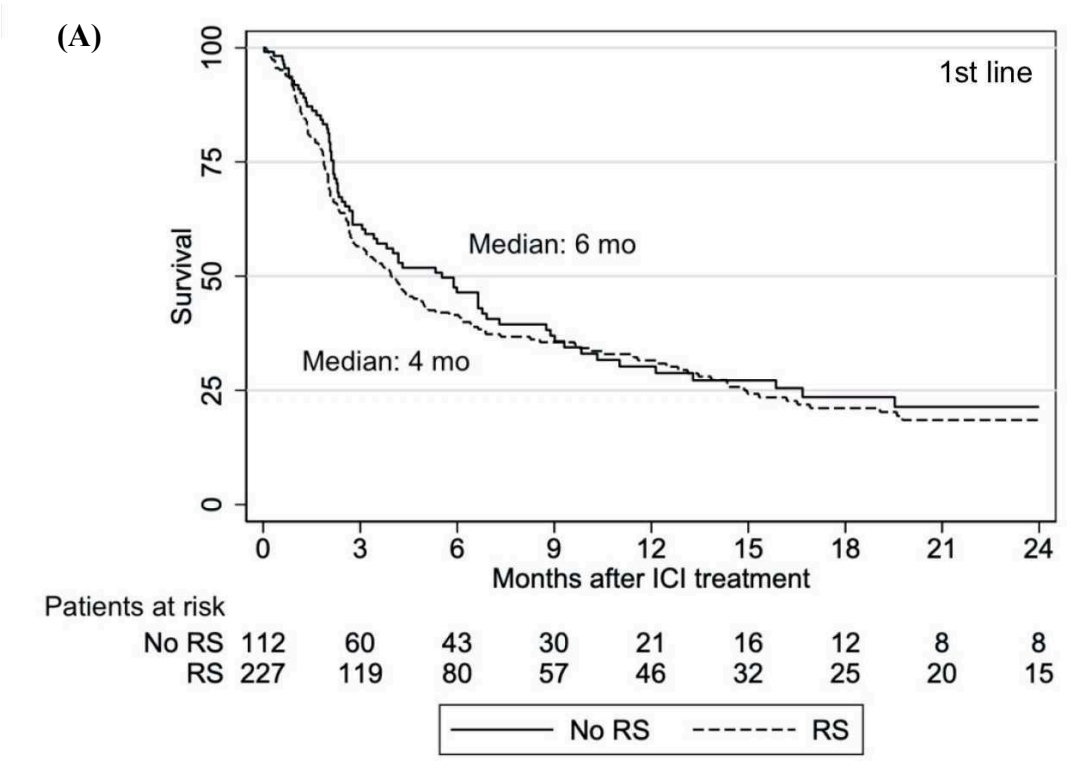
[a]Adjusted for internally developed risk score [11] for first line and Bellmunt score [16] for subsequent line

[b]p-value<0.05

Abbreviations: ORR: Observed Response Rate, OR: odds ratio, OS: Overall Survival; PFS: Progression-Free Survival, HR: Hazard Ratio; CI: Confidence Interval

Figure 2. Kaplan Meier curves for progression-free survival (PFS) with checkpoint inhibitors in 1st line (A) and 2nd+ line (B) setting

(RS: Radical Surgery, ICIs: Immune Checkpoint Inhibitors)



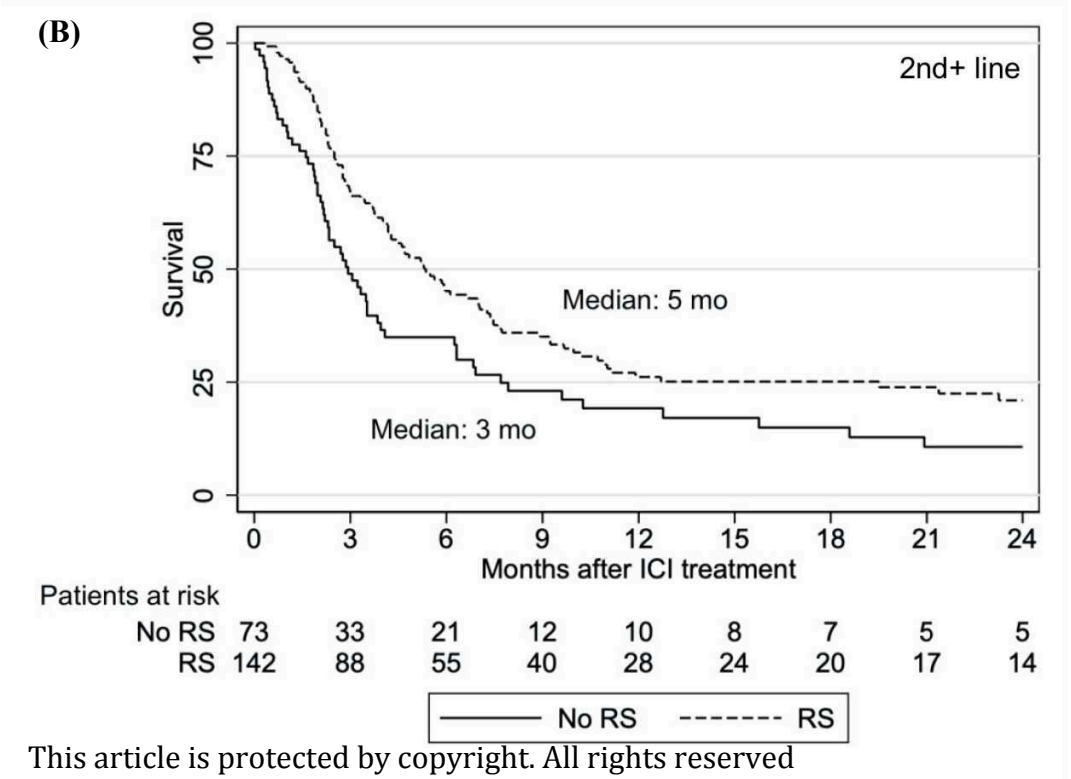


Figure 3. Kaplan Meier curves for overall survival (OS) with checkpoint inhibitors in 1st line (A) and 2nd+ line (B) setting

(RS: Radical Surgery, ICIs: Immune Checkpoint Inhibitors)