Androgen Receptor Expression Is a Predictor of Poor Outcome in Urothelial Carcinoma

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Background: Several preclinical and clinical studies demonstrated a strong correlation between androgen receptor (AR) signaling and bladder tumorigenesis. This study aims to evaluate the prognostic significance of AR expression in metastatic urothelial carcinoma (mUC).

Methods: Samples from a phase III trial (LaMB, NCT00949455) which compared maintenance lapatinib versus placebo after completion of first-line platinum-based chemotherapy in patients with HER 1/2-positive mUC of the bladder were collected. Corresponding baseline and follow-up data included patients enrolled in the study and those who screen failed. AR expression was assessed independently by a single pathologist who was blinded to the study. Samples were grouped according to AR expression (negative vs. positive) and correlated with baseline tumor characteristics and survival.

Results: Of the 446 screened samples in the LaMB study, 90 were retrospectively analyzed for AR expression. There were no correlations between AR expression and tumor stage ($r = -0.10$), tumor grade ($r = 0.05$) at diagnosis, or subsequent treatment with lapatinib ($r = -0.04$). The median progression-free survival was 6 months (95% CI, 3.20–6.80) in the AR-negative group and 5 months (95% CI, 3.41–6.59) in the AR-positive group [HR 0.54 (95% CI, 0.31–0.92), $p = 0.02$]. Similarly, patients with AR-negative disease had more favorable overall survival (OS) with 16 months (95% CI, 6.6–25.4) and 11 months (95% CI, 7.0–15.0) in the AR-positive group [HR 0.55 (95% CI, 0.31–0.98), $p = 0.04$]. In the multivariate analysis, AR expression was significantly associated with worse OS ($p = 0.045$).

Conclusions: AR expression is a predictor of poor outcome and presents a targetable alteration in patients with mUC.

Keywords: androgen receptor, AR, urothelial carcinoma, bladder cancer, survival

INTRODUCTION

Although the exact mechanism of urothelial carcinoma development remains unknown, increasing evidence suggests that the androgen receptor (AR) signaling pathway plays a potential role (1). While traditionally not thought to be an androgen-driven malignancy, urothelial carcinoma is associated with a strong male preponderance, and preclinical studies demonstrated an association between AR signaling and the development and progression of urothelial carcinoma (1–3). Furthermore, The Cancer Genome Atlas (TCGA) bladder cancer analysis showed that while somatic alterations in the AR gene are rare (4), a higher expression of genes involved in AR signaling was observed in luminal tumors (5). Together, these findings suggest that AR could be a rational therapeutic target in bladder cancer.

Here, we performed AR staining on prospectively collected tissue samples of patients with metastatic urothelial carcinoma (mUC) of the bladder treated with first-line platinum-containing chemotherapy and assessed AR expression and its prognostic significance.

PATIENTS AND METHODS

Tissue samples from a phase III, randomized, double-blind trial (LaMB) which compared maintenance lapatinib versus placebo after completion of first-line platinum-based chemotherapy in patients with human epidermal growth factor receptor 1/2 (HER1/2)-positive mUC of the bladder (NCT00949455) were prospectively collected. Patients with metastatic upper tract urothelial carcinoma or other concomitant malignancies were excluded from the study. A total of 446 patients were screened for HER1/2 expression. Those who tested positive and obtained disease control or response after first-line chemotherapy (4–8 cycles) were randomly assigned to lapatinib or placebo (1:1). Tissue and the corresponding baseline and follow-up data were collected both from patients enrolled in the study and those who screen failed due to either disease progression or biomarker-negative disease.

The study did not meet its primary endpoint of progression-free survival (PFS) [HR 1.07 (95% CI, 0.81–1.43), $p = 0.63$)] or secondary endpoint of overall survival (OS) [HR 0.96 (95% CI, 0.70–1.31), $p = 0.80$)]; thus, the addition of maintenance lapatinib did not contribute to outcomes compared with standard of care only. (6) Of the 446 screened tumor samples in the LaMB study, 90 were retrospectively analyzed for AR expression and correlated with treatment outcome. All patients gave informed consent for translational research as part of the study, which had appropriate ethical approval (07/H1102/73).

Archived, paraffin-embedded tissue blocks from patients with histologically confirmed urothelial carcinoma of the bladder were used. Androgen receptor immunohistochemistry (IHC) staining was performed on 4-μm-thick sections, using the clone AR441 (Dako, California, USA). Scoring was performed independently by a single pathologist who was blinded to the study. The intensity of staining was graded as IHC 0 to 3+. Any amount of staining was considered positive, and an average was used for the final score for each patient. Samples were stratified according to AR expression and divided into two groups: AR negative (IHC 0) and AR positive (IHC 1+, weakly positive; 2+, moderately positive; 3+, strongly positive) (7).

Data from patients with mUC who were screened in the LaMB study were used to determine baseline characteristics. Progression-free survival was defined as the time from the end of first-line chemotherapy until disease progression or death due to disease, following the primary endpoint of the study. Overall survival was defined as the time between the end of chemotherapy and death of any cause. Patients alive at the date of last disease assessment were censored. Progression-free survival and OS were compared using the log-rank test stratified by AR expression levels, and the corresponding two-sided 95% CIs were presented. Multivariate analysis was conducted to correlate clinical and pathological factors with PFS and OS. Correlations between AR expression and tumor or treatment characteristics were measured by Pearson product-moment correlation coefficient. p-values of ≥ 0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0.

RESULTS

In total, 90 samples from patients with mUC of the bladder were stained for AR expression (**Figure 1**). Of these, 79% (71/90) were men, with a median age of 71 years (IQR 66–78). Seventy-five percent (68/90) had an ECOG performance status of 0–1 at the time of diagnosis and 35% (32/90) had visceral metastatic disease (**Table 1**). Patients received standard-of-care first-line chemotherapy mainly with platinum-containing regimens. Among the 90 patients, 12% (11/90) had disease progression on first-line chemotherapy and screen failed in the LaMB study. A furthe0r 27% (24/90), though benefiting from chemotherapy, screen failed due to not meeting all eligibility criteria of the study. The remaining 61% (55/90) of patients achieved either response or stable disease on first-line chemotherapy and were enrolled in the LaMB study. Of these, 53% (29/55) of patients were randomized to the lapatinib maintenance therapy and the remaining 47% (26/55) of patients received placebo.
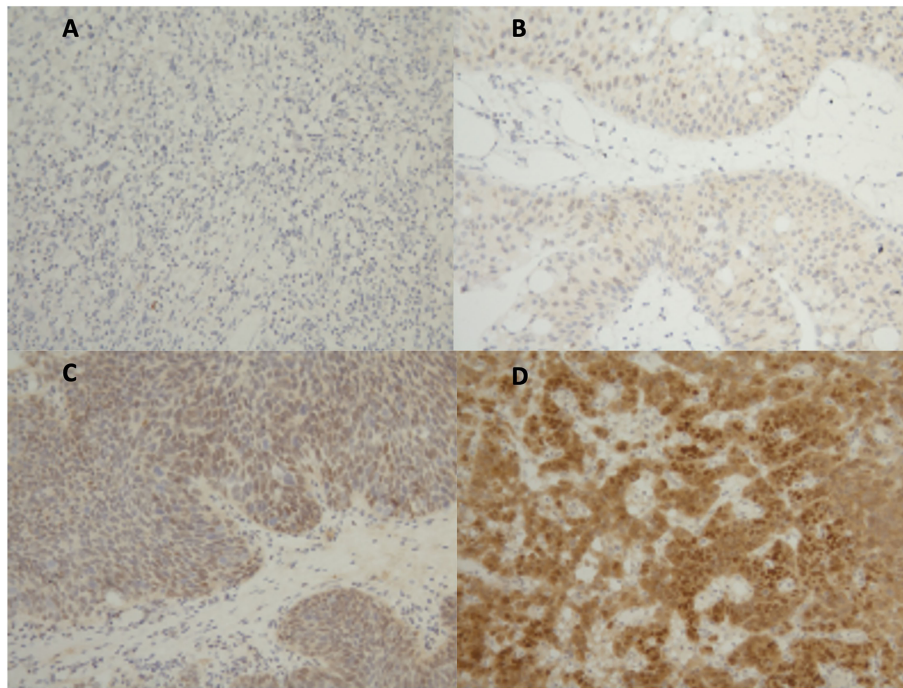
FIGURE 1 | Androgen receptor immunohistochemistry (IHC) staining. **(A)** IHC negative; **(B)** 1+, weakly positive; **(C)** 2+, moderately positive; **(D)** 3+, strongly positive.

Among the 90 stained samples, 62% (56/90) were AR negative and 38% (34/90) demonstrated AR expression at various intensities. Positive AR expression rates in samples originating from male and female patients were found in 38% (27/71) and 37% (7/19) of cases, respectively. There were no correlations between AR expression and tumor stage ($r = -0.10$), tumor grade ($r = 0.05$) at diagnosis, or subsequent treatment with lapatinib ($r = -0.04$).

Next, we explored the relationship between AR expression and survival. The median PFS from the end of chemotherapy was 5 months (95% CI, 3.8–6.2) and the median OS was 13 months (95% CI, 8.1–17.8) for the whole cohort. The median PFS was 6 months (95% CI, 3.20–6.80) in the AR-negative group and 5 months (95% CI, 3.41–6.59) in the AR-positive group (**Figure 2A**). The hazard ratio for disease progression or death from disease was 0.54 (95% CI, 0.31–0.92; $p = 0.02$) (**Figure 2A**). Similarly, patients with AR-negative disease had a more favorable OS with 16 months (95% CI, 6.6–25.4) in the AR-negative group and 11 months (95% CI, 7.0–15.0) in the AR-positive group (**Figure 2B**). The hazard ratio for death was 0.55 (95% CI, 0.31–0.98; $p = 0.04$) (**Figure 2B**).

Subgroup analysis showed no associations between the intensity of staining (IHC 1+, 2+, 3+) and PFS ($p = 0.13$). However, samples with moderately (IHC 2+) and strongly positive (IHC 3+) AR expression ($n = 19$) were associated with worse OS [7 months (95% CI, 3.12–10.88), $p = 0.014$]. In the multivariate analysis, no factors were significantly associated with better PFS. Androgen receptor expression was significantly associated with worse OS ($p = 0.045$).

DISCUSSION

In recent years, there has been a significant progress in the treatment landscape of mUC with the advent of immune checkpoint inhibitors and antibody–drug conjugates. Compared with other solid tumors, (8) there is a lack of targeted therapy options. The only currently approved treatment is erdafitinib, a pan-fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor (9); however, only 20% of patients with advanced urothelial carcinoma have FGFR alterations (10). Furthermore, targeted therapy options are necessary to improve the prognosis of patients with mUC.

In this retrospective study, we showed that AR expression is a predictor of poor outcome in patients with mUC receiving first-line platinum-containing chemotherapy. Both PFS and OS were poorer in the AR-positive patient group [HR 0.54 (95% CI, 0.31–0.92), $p = 0.02$ and HR 0.55 (95% CI, 0.31–0.98), $p = 0.04$, respectively].

Several preclinical studies demonstrated a link between AR expression and the development of bladder cancer, especially in men (3, 11, 12). A recent meta-analysis of immunohistochemical studies including 2,049 patients from 13 retrospective studies showed a strong correlation between AR expression and gender

TABLE 1 | Baseline patient characteristics.

Patient characteristics	All patients (n = 90)	AR positive (n = 34)	AR negative (n = 56)
Age (years), median (IQR)	**71 (66–78)**	**69 (64–77)**	**73 (66–78)**
Gender, n (%)			
Male	71 (79)	27 (79)	44 (79)
Female	19 (21)	7 (21)	12 (21)
ECOG PS, n (%)			
0	28 (31)	11 (32)	17 (30)
1	40 (44)	15 (44)	25 (45)
>1	8 (9)	3 (5)	5 (9)
NK	14 (16)	5 (9)	9 (16)
T stage, n (%)[a]			
Ta	1 (1)	1 (3)	0 (0)
T1	7 (8)	2 (6)	5 (9)
T2	30 (33)	11 (30)	19 (35)
T3	31 (34)	15 (42)	16 (30)
T4	15 (17)	3 (8)	12 (22)
NK	6 (7)	2 (11)	4 (4)
Tumor grade, n (%)[a]			
Grade 2	5 (6)	3 (9)	2 (4)
Grade 3	81 (90)	29 (85)	52 (92)
NK	4 (4)	2 (6)	2 (4)
Visceral metastasis, n (%)[b]			
Yes	32 (35)	13 (38)	19 (34)
No	52 (58)	19 (56)	33 (59)
NK	6 (7)	2 (6)	4 (7)
First-line chemotherapy, n (%)			
Cisplatin/gemcitabine	50 (56)	22 (65)	28 (50)
Carboplatin/gemcitabine	27 (30)	8 (24)	19 (34)
Other[c]	13 (14)	4 (12)	9 (16)
Response to previous chemotherapy, n (%)			
CR/PR	47 (52)	17 (50)	30 (54)
SD	30 (34)	13 (38)	17 (30)
PD	11 (12)	3 (9)	8 (14)
NK	2 (2)	1 (3)	1 (2)
Received lapatinib maintenance therapy, n (%)	29 (32)	9 (10)	20 (22)

IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group (ECOG) Performance Status; NK, not known; CR/PR, complete response/partial response; SD, stable disease; PD, progressive disease.
[a]Tumor stage and tumor grade at first diagnosis of urothelial carcinoma.
[b]Presence of visceral metastasis at the start of first-line chemotherapy.
[c]Other chemotherapy regimens included oxaliplatin, Mopq10, vinflunine, and gemcitabine.

(male vs. female: OR = 0.658; p = 0.027) or tumor grade (low grade vs. high grade: OR = 0.575; p < 0.001) (13). Our study showed no correlation between AR expression and baseline tumor stage or grade which is in line with previously published conflicting data (14).

Another study compared AR expression in normal urothelium and urothelial carcinoma tissue and demonstrated that AR-positive tumors had a poorer prognosis than those with no AR expression (15). We observed similar findings with AR-positive samples demonstrating significantly shorter PFS and OS. These results indicate that AR expression is a predictor of poor outcome in mUC. Further work is needed to explore these findings.

A rising number of therapeutic agents targeting AR are approved or under development, making these preliminary findings clinically relevant. A systematic review of preclinical and clinical studies evaluating the impact of AR modulation on outcomes of urothelial carcinoma demonstrated that AR modulators have the potential to interfere with the activity of multiple anticancer agents used for the treatment of urothelial carcinoma. Moreover, the relative risk of non-muscle-invasive bladder cancer recurrence is significantly lower in patients undergoing therapy with five alpha reductase inhibitors or androgen deprivation therapy [RR 0.50 (95% CI, 0.30–0.82), p = 0.006] (16).

The limitations of our study include the retrospective data analysis and the relatively small number of patients with AR-positive disease. Furthermore, data were analyzed from the completion of chemotherapy in line with the maintenance approach of the study. Maintenance targeted therapy is not standard of care, thus justifying this approach. Nevertheless, the effects from a more orthodox frontline perspective are unknown. A large, prospective randomized study may be justified to validate these findings and potentially repurpose AR-modulating agents in this context.
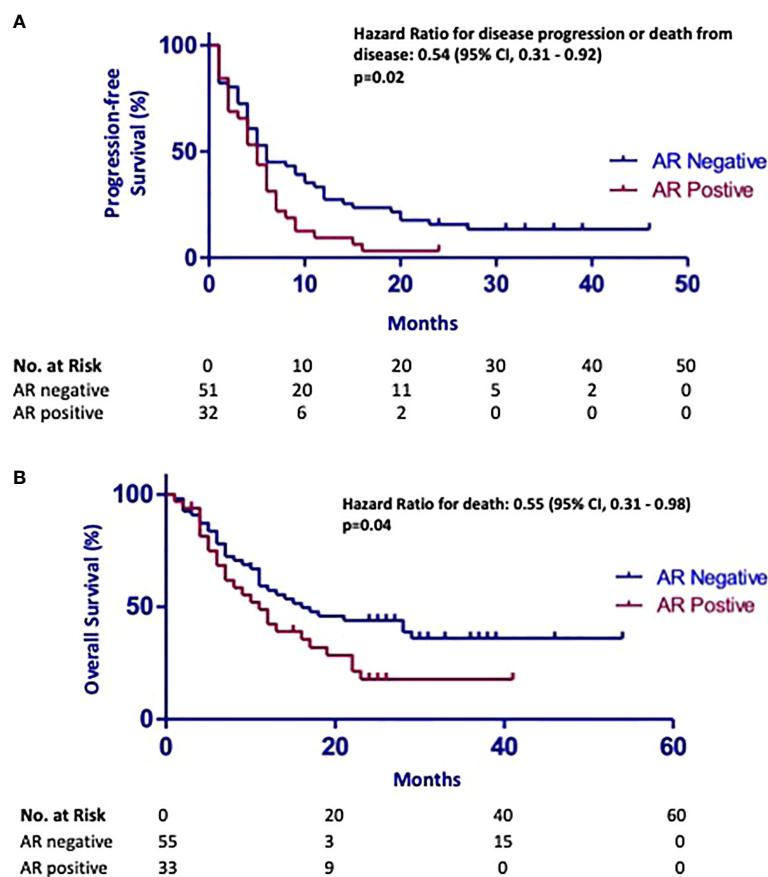
FIGURE 2 | **(A)** Kaplan–Meier curves demonstrating that high expression of androgen receptor (AR) in metastatic urothelial carcinoma is associated with shorter progression-free survival [HR 0.54 (95% CI, 0.31–0.92), p = 0.02]. **(B)** Kaplan–Meier curves showing that high expression of androgen receptor (AR) in urothelial carcinoma samples is associated with poor overall survival [HR 0.55 (95% CI, 0.31–0.98), p = 0.04].

CONCLUSION

Androgen receptor expression is a predictor of poor outcome in mUC and presents a targetable alteration in bladder cancer. Further exploration is needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The original study involving human participants were reviewed and approved by London – City and East (07/H1102/73). The patients/participants included here provided their written informed consent to participate in future research

AUTHOR CONTRIBUTIONS

CA: conceptualization, methodology, validation, writing—original draft, and visualization. BS: conceptualization and writing—original draft. SD, JC, FJ-S, and CT: data curation and visualization. GT and DB: formal analysis. TP: supervision. All authors read and approved the final version of the manuscript.

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