Editorial Commentary

Are primary tumors suitable for biomarker-guided treatment of metastatic urothelial cancer?

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In recent years, the therapeutic armamentarium for patients with metastatic urothelial carcinoma (mUC) has expanded with the emergence of novel therapies like immune checkpoint inhibitors (ICIs), tyrosine kinase inhibitors (TKIs) targeting fibroblast growth factor receptors (FGFRs), and antibody-drug conjugates (ADCs). Although FGFR inhibitors are reserved for patients with FGFR altered urothelial carcinoma (UC), accurate predictive biomarkers for these new drugs that correlate to individual patient responses, are lacking. Biomarker-guided response prediction on primary tumor samples is appealing due to the availability of archived tumor material typically present at the diagnosis of mUC. Additionally, the bladder is easy to access to obtain tumor biopsies necessary for biomarker assessment, whereas fine needle aspiration or biopsy from distant metastatic sites can be technically challenging. However, primary bladder tumors might not be representative of metastatic lesions, as the stability of UC phenotypes during progression to metastatic disease remains elusive.

This poses an important limitation that needs to be addressed before the implementation of biomarker-guided treatment strategies. Biomarkers predictive of response may be present in primary bladder tumors but absent in metastatic lesions, potentially leading to ineffective treatment. The other way around is also possible, as a result of acquired disease features over time. We therefore appreciated reading the study "Molecular Urothelial Tumor Cell Subtypes Remain Stable During Metastatic Evolution" and want to commend the authors for their valuable findings (1). Cox *et al.* performed immunohistochemistry (IHC)-based molecular subtyping on 138 primary UC and matched mUC samples, and discovered luminal, basal, and neuroendocrine molecular subtypes to remain relatively stable during metastatic evolution (94% concordance between matched pairs). In contrast, transcriptome-based muscle-invasive bladder cancer (MIBC) consensus subtyping on a subset of matched primary (N=20) and metastatic (N=20) samples proved to be highly variable (45% concordance).

Prior genetic studies comparing primary and matched mUC samples, showed early evolutionary branching of metastatic tumor clones, with limited mutations shared between the metastatic lesions and the bulk of the

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primary tumor (2,3). Noteworthy, potentially actionable gene mutations [*FGFR3*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), TSC complex subunit 1 (*TSC1*), Erb-B2 receptor tyrosine kinase 2 (*ERBB2*)] have been reported to be discordant in up to 23% of primary and metastatic tumor pairs (4). Intratumoral heterogeneity and sampling bias contribute to this genetic discordance, exemplified by the enrichment of *FGFR3* mutations in superficial areas of transurethral resection of bladder tumor (TURBT) samples compared to more invasive regions of the tumor (5). Taken together, evidence suggest that primary tumor samples might not be representative of metastatic lesions. High phenotypic concordance at the protein level observed by Cox *et al.*, however, demonstrated at least partial phenotypic stability during tumor evolution and metastatic dissemination, despite patients undergoing different systemic treatments between sequential samples. The observed similarity between primary tumors and metastatic lesions warrants scientists not to exclude primary tumors from efforts investigating novel biomarkers.

Bulk RNA sequencing-based consensus cluster (CC) molecular subtypes provide a snapshot of the tumor-microenvironment (TME), and might not be optimal for characterizing metastatic lesions of UC (6,7). Briefly, MIBC consensus subtypes (8) were designed for the classification of primary tumors and lean heavily on stromal content (e.g., luminal infiltrated, stroma-rich). The applicability of CCs to characterize and compare different metastatic lesions with diverse associated TMEs is therefore limited, and site-specific classification systems might be more appropriate (9). Additionally, interpretation of CCs applied to post-treatment samples must be performed with care, as surgical or systemic pre-treatment induces scar-like phenotypes associated with fibroblasts and the stroma-rich subtype (10,11). Subtype switches between TURBT and radical cystectomy (RC) tumor samples observed in earlier studies were often centered around the stroma-rich subtype, as is the case for the majority of discordant samples observed by Cox *et al.* (8 out of 11). Since the majority of primary tumors analyzed by Cox *et al.* was derived at RC it is difficult to assess if the observed discordance between primary and metastatic samples truly reflected naturally occurring metastatic tumor evolution or simply highlights treatment effects induced by local surgery. Nonetheless, there is currently no role in routine practice for molecular subtype determination in UC (12).

Biomarkers that are used in current clinical practice are programmed cell death ligand 1 (PD-L1) expression on immune cells (ICs) and the combined positive score (CPS), which are IHC-based companion diagnostics designed to guide ICI in mUC patients (12). A study evaluating PD-L1 expression and CPS scores between matched primary tumors and metastatic lesions suggested that tumor cell PD-L1 expression and CPS positivity tended to decrease during metastatic progression (13). A similar observation was reported in a small study evaluating PD-L1 expression in primary tumors and matched liver metastases (14). While PD-L1 expression remained stable on tumor cells, the presence of tumor infiltrating lymphocytes (TILs) and accompanying PD-L1 expression on TILs was significantly lower in liver metastases than in matched primary tumors (14).

Consistent with these findings, two separate studies reported around 75–80% concordance of PD-L1 expression when comparing primary and matched metastatic samples (15,16). Concordance of PD-L1 expression was significantly lower in patients treated with pre-operative chemotherapy (15), while concordance rates observed between synchronous and metachronous primary and matched metastatic pairs did not significantly differ (16). However, as most samples have low IC PD-L1 positivity and/or CPS, the inherent likelihood of concordance between primary and metastatic samples is relatively high. Consequently, future research should prioritize investigating the predictive value of biomarkers in both primary and metastatic samples to anticipate patient responses to treatment.

With the emergence of ADCs, target proteins like NECTIN-4 [enfortumab vedotin (EV)] and TROP2 [sacituzumab govitecan (SG)] are upcoming markers of particular interest. The phase I EV-101 study, evaluating EV in patients with solid tumors, has been amended during the trial duration because the majority of patients with UC exhibited high levels of NECTIN-4 tumor staining and this eligibility requirement was removed (17). This modification allowed for a broader exploration of EV response, albeit without a specific focus on NECTIN-4 expression. In the EV-201 study, a phase 2 single-arm open label study of EV in either third line cisplatinum-eligible patients or second line in cisplatinum-ineligible UC patients, NECTIN-4 expression was examined via IHC. NECTIN-4 expression was not an eligibility criterion, however, in this study its range of expression was fairly broad (18). Preclinical studies on TROP2 showed high protein expression of TROP2 in UC, and as a result, TROP2 expression was not an inclusion criterion in TROPHY-U-01, the phase 2 single-arm trial evaluating SG in UC patients (19,20).

Disparate expression of NECTIN-4 and TROP2 has been shown in different histological subtypes (21), although data are limited. UCs can have variable and mixed histology in 25–30% of cases (22), comprising a substantial part of the patient population. For molecular subtypes, NECTIN-4 shows a high level of expression mainly in luminal-a UC (9,21), whereas TROP2 mRNA expression is consistently high across molecular subtypes, except for the neuroendocrine subtype (23). A recent study reported decreased NECTIN-4 expression levels in primary versus matched metastatic UC, with 40% of metastatic lesions lacking membranous NECTIN-4 protein expression (24). In contrast, a correlation between TROP2 mRNA levels and TROP2 protein expression (IHC) was observed and, there was no apparent decrease of TROP2 mRNA expression in metastatic samples when compared to the paired primary tumor. Taken together, it is of importance to recognize that expression levels of membranous targets are affected by intra- and inter-patient heterogeneity and disease evaluation overtime.

While ADC effectivity can be affected by several factors, e.g., ADC composition, target expression, intra-tumoral drug penetration, and composition of ICs and stromal cells in the tumor micro-environment, thus so far, no clear predictor of patient response to ADC has been identified, with the relation between target expression and drug efficacy still being unresolved. A relationship between target expression level and degree of ADC activity seems to be logical, although, target protein expression based on IHC may not be the best candidate for patient selection. Membranous NECTIN-4 expression was associated with survival benefit in 47 EV treated patients, with 21 of 47 cases being assessed in metastatic lesions (24). Prospective trials are needed to identify predictive biomarkers for ADC efficacy, including the evaluation in matched primary and metastatic lesions.

To conclude, consistent protein-expression levels of urothelial differentiation markers observed between primary and metastatic UC suggest that biomarkers can be assessed on (archived) primary tumor material, omitting the need for target metastatic lesion biopsies and associated treatment delay for some patients. Prior to clinical implementation, the predictive value of novel biomarkers should be prospectively evaluated and reported separately for primary tumors and metastatic target lesions. An illustrative example is the THOR trial, wherein cohort I randomized patients with *FGFR2* or *FGFR3* alterations to either erdafitinib (an FGFR inhibitor) treatment or the investigator's choice of 3rd line chemotherapy (25). The authors stated that most alterations were evaluated on archived tumor tissue but did not report whether examining fresh metastatic biopsies or archived primary tumor material resulted in different patient outcomes. This overlooks a chance to gain valuable insights into the utilization of archived primary tumor samples for treatment selection of mUC patients whom often receive multiple lines of therapy between initial diagnosis and the start of 3rd line therapy.

With current evidence in mind, it seems that presence of biomarkers in primary tumors often does not reflect their status in metastatic lesions. Consequently, conducting biomarker assessment on primary tumors to guide treatment of mUC patients should be approached with care.

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