Editorial

Liquid biopsies in bladder cancer—did we find the Holy Grail for biomarker analyses?

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Provenance: This is a Guest Editorial commissioned by Editorial Board Member Dr. Xiongbing Zu, MD, PhD (Department of Urology, Xiangya Hospital, Central South University, Changsha, China).

Comment on: Birkenkamp-Demtröder K, Nordentoft I, Christensen E, *et al.* Genomic Alterations in Liquid Biopsies from Patients with Bladder Cancer. Eur Urol 2016;70:75-82.

Submitted Jul 24, 2016. Accepted for publication Jul 25, 2016.
doi: 10.21037/tau.2016.09.03
View this article at: http://dx.doi.org/10.21037/tau.2016.09.03

The natural history of urothelial carcinoma of the bladder (UCB) and its treatment strategies are highly variable. While some patients never experience disease recurrence, others experience disease progression and eventually decease of their disease. Indeed, UCB is not only clinically, but also genetically a highly heterogeneous disease. Phenotypically similar tumors may harbor completely different molecular genotypes representing the individuality of each tumor and its host. Biomolecular predictors hold the potential to unmask individual genomic, epigenetic, transcriptomic, and proteomic alterations that may explain the variable clinical course of disease (1).

Contemporary histopathological workup and molecular diagnostics in cancer patients is typically based on tissue biopsies of the primary tumor or the metastatic site. Individual tumors, however, mostly consist of diverse subpopulations, and the small amount of tissue obtained by biopsy may not unconditionally represent the most aggressive subclone (2). In the era of personalized medicine, however, there is an urgent need for robust non-invasive biomarkers to optimize patient counseling and treatment individualization (3). In UCB three sources for biomarker analyses exist: tissue, urine and blood, respectively. The recently published study entitled "Genomic Alterations in Liquid Biopsies from Patients with Bladder Cancer" by Birkenkamp-Demtröder *et al.* published in *European Urology* is addressing this controversy (4).

The authors of this retrospective study investigated non-invasive disease monitoring in non-muscle invasive bladder cancer (NMIBC) patients. In total, 377 samples consisting of tumor tissue, and "liquid biopsies" from plasma and urine from 12 patients with recurrent or progressive UCB were analyzed by various next-generation sequencing methods, to identify genomic variants in tumor-specific DNA. For disease monitoring, the authors designed one to six highly sensitive tumor-specific personalized assays using digital PCR. They found that particularly patients with disease progression to muscle invasive or metastatic bladder cancer had increased inter-tumor heterogeneity. After 4 to 20 years follow-up, tumor-specific cell-free DNA levels in plasma and urine prior to disease progression were significantly elevated in patients with disease progression, compared to patients with recurrent NMIBC. In urine, high levels of tumor DNA were detected in all patients with progressive disease, compared with low levels in samples from patients with recurrent disease. Tumor DNA was undetectable in patients without disease recurrence or progression. The authors conclude that tumor DNA may represent a promising genomic biomarker for disease progression, and personalized assays of genomic variants may allow non-invasive disease surveillance in the future.

The term "liquid biopsy" is usually used for blood-based analysis of circulating tumor cells (CTC) and cell-free circulating nucleic acids [e.g., circulating tumor DNA

(ctDNA)] released into the peripheral blood from the primary tumor and/or metastatic deposits (2). The authors of this study extended the definition of liquid biopsies, as they developed personalized assays for disease surveillance based on individual tumor-specific genomic variants using liquid biopsy specimens from the blood and urine. As previous studies have demonstrated, urine may frequently contain epitopes and genetic material from current or previous UCB (5). Intuitively it feels reasonable to combine information obtained from liquid biopsies from the blood and urine in UCB patients, as genomic characteristics may vary between the primary tumor site and distant sites (6). In addition, urine is the medium the tumor is in close contact to and thus, tumor cells may be released to this source early at time of local recurrence. Therefore, the authors should be congratulated for this endeavor.

It is of utmost importance to understand the molecular pathways underlying the clinical behavior of UCB. Fundamental research over the last decades has provided vital insight into the molecular pathogenesis of UCB and has the potential for improving clinical decision-making and treatment (7). It has become clear that UCB develops along complex molecular pathways, and that different steps such as tumor initiation, progression, and metastatic propensity are closely linked to a variety of genetic and epigenetic events (1). Molecular alterations may occur as a single event, but an augmented tumor biologic aggressiveness influencing carcinogenesis and progression is likely to be associated with multiple alterations in serial, parallel, and complementary pathways (8). Among all solid cancers, UCB is definitely one of the malignancies with the highest frequency of mutations (9,10). Birkenkamp-Demtröder and colleagues confirmed a significant level of heterogeneity in UCB and also reported variable findings between different samples in their study (4).

Due to the multitude of genomic alterations, the permanent search for the optimal UCB biomarker somehow represents a search for the Holy Grail. Indeed, the identification of significant recurrent mutations in several genes including multiple genes involved in cell-cycle regulation, chromatin regulation, and kinase signaling pathways suggest new possibilities for bladder cancer treatment (9). Concentrated efforts of several research groups have tried to translate this new understanding from the bench to the bedside, but yet only very few molecular biomarkers have been implemented in clinical routine UCB diagnostics. An important limitation is that most single molecular markers do not provide sufficient information to be used independent of other clinical information. Tumor-specific personalized molecular assays as used by Birkenkamp-Demtröder *et al.* may eventually represent a valid approach for individualized outcome prognostication and treatment surveillance. However, an optimal molecular marker or marker panel does not only have to answer a clinically relevant question and provide information that is not available in a more simple way. Any new molecular diagnostic tool also needs to provide a benefit over standard criteria or at least improve their accuracy in a cost-effective fashion (11). Thus, the optimal use of molecular markers is most likely the application of marker panels incorporated into a model combined with standard clinical data (8).

In their paper, the authors investigated the association of ctDNA of plasma and urine with UCB outcomes. They found higher levels of ctDNA in plasma and urine before disease progression, compared with patients with recurrent disease. Moreover, ctDNA was no longer detectable in disease-free patients, who were treated for non-invasive disease. ctDNA is single- or double-stranded DNA released from necrotic and apoptotic tumor cells, metastasis or CTC into the blood and thus harbors the mutations of the original tumor (12). An important challenge of this technology is the identification of DNA that unequivocally derives from UCB in contrast to cell-free DNA (cfDNA) that is released by dying non-malignant host cells. Obviously, normal cfDNA dilutes the ctDNA in patients with cancer and thus hampers their detection, particularly in situations with increased tissue damage (e.g., surgery, chemotherapy, or radiotherapy) (13). The authors of this study used whole genome sequencing, whole exome sequencing, and mate-pair sequencing as well as polymerase chain reaction to identify and analyze genomic variants of the tumor and matched germline DNA. Despite significant improvements of these highly sensitive and specific modalities in the past decades, the clinical use of liquid biopsies using ctDNA has not been implemented for routine clinical practice for several clinical and technical reasons. Most notably, the lack of standardization and automation of the technology and variability in molecular DNA assays results in significant intra- and inter-laboratory and observer differences (14). In contrast, standardized and semiautomated systems for immunocytologic and molecular detection of CTC are commercially available. CTC are viable tumor cells leaving actively the primary tumor and/or metastasis (15). Compared to ctDNA, CTC detection and capturing holds the potential of complete DNA, RNA (mRNA/microRNA), and protein functional studies. Previous studies on CTC in

UCB have found that this biomarker is a strong predictor for disease recurrence, disease progression, cancer-specific and overall death, respectively, in patients treated with bladder sparing surgery (16) or radical cystectomy (17,18). In addition, CTC are also associated with inferior survival in patients with metastatic UCB (19). The results of this and previous studies strongly suggest integrating ctDNA or CTC in future UCB clinical trials to shed more light on the potential of this promising biomarker. The findings of Birkenkamp-Demtröder *et al.* (4) support the role of liquid biopsies for UCB screening and early cancer detection. Moreover, they underscore the value of liquid biopsies in real-time monitoring of disease, therapeutic effect and need of intervention.

Interestingly, the authors found that ctDNA was detected even in the plasma of NMIBC patients, although Ta tumors should have an intact basal membrane (4). Indeed, this finding is in congruence with observations in other cancer entities, in which ctDNA also was found in early tumor stages (20) and thus may represent an interesting and important target for future investigations. Up to quarter of patients with high-risk NMIBC will decease of their disease after bladder-sparing treatment (21), which is a disproportional high rate for early stage tumors and may be due to occult micrometastatic disease undetectable with conventional cross-sectional imaging. We and other investigators previously also reported the importance of CTC in early bladder cancer (16-18,22). Interestingly, ctDNA seems to be more frequently present in patients with early disease stages without detectable CTC (13,15). However, not all patients with presence of ctDNA or CTC in NMIBC will experience disease progression or metastatic disease. Future investigations therefore also have to address the process of cancer dormancy and identify representative, clinical relevant ctDNA or CTC clones that harbor the risk to progress to clinical significant disease.

In conclusion, there is increasing evidence, that liquid biopsies are a useful companion for ctDNA and CTC analyses in UCB and may help in screening and early detection of UCB patients at risk for disease recurrence and progression. The current study underscores the potential value of liquid biopsies for real-time monitoring of disease and therapy. The complex underlying genetic heterogeneity of UCB is challenging to move from bench to bedside. Individualized molecular assays may facilitate sensitive and specific ctDNA identification, but standardized, automated CTC detection tools allow an in-depth assessment of viable tumor cells at various levels. Importantly, ctDNA and CTC should be recognized as different and complementary, not competitive biomarker (23). Despite the enormous potential of these biomarkers, further resolving of technical limitations and investigation in prospective, randomized clinical UCB trials are inevitable to impel this biomarker from fiction to fact.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Rink M, Shariat SF, Soave A. Liquid biopsies in bladder cancer—did we find the Holy Grail for biomarker analyses? Transl Androl Urol 2016;5(6):980-983. doi: 10.21037/tau.2016.09.03

