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Clinical utility versus futility: a tipping point for liquid biopsies in bladder cancer

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“Liquid biopsy based biomarker may find use in risk stratification, improvement in recurrence metastatic detection, assessment of treatment response and prediction of treatment efficacy”

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Highlights

With the emergence of genomic profiling and the drive toward targeted molecular therapies, cancer-specific biomarkers have undergone rapid concurrent development for use in precision-based medicine. From a basic standpoint, such biomarkers represent objectively measured biomolecules such as DNA, RNA, protein or peptide modifications within normal or abnormal biological states. Novel biotechnologies have shed insight into hitherto unknown genomic and proteomic alterations and potential techniques for their detection, isolation and interpretation. In addition, there has been an expansion of the fundamental genetic understanding of perturbed biological states within clinical oncology. These critical molecular and cellular biomarkers of such aberrations represent the hallmark of cancer and formulate the foundation of the multitude of studies aimed at change in clinical practice [1–3].

Characterization of tumor-derived cell-free DNA (ctDNA) found in blood samples is termed liquid biopsy and represents a significant technological advancement with potential clinical applications [4–7]. To date, decisions for management of urothelial carcinoma of the urinary bladder (UCB) is based upon traditional pathological characteristic features of the primary tumor radiological findings for initial staging and detection of subsequent metastases and/or recurrence which is suboptimal and fraught with inherent limitations [8–10]. As molecular characterization of tumors has become increasingly important toward understanding factors impacting the clinical management of UCB, ctDNA has become increasingly attractive as a biomarker with potential means to facilitate the diagnosis, treatment selection, monitoring treatment response, tracking minimal disease and treatment resistance [11].

The ‘tipping point’ is a term used to describe the moment in time in which incremental increases in technological understanding culminates in an expansion of knowledge which creates change. For molecular diagnostic testing, this ‘tipping point’ represents the transformation of fundamental genetic discovery into application and subsequent utilization for use in the improved clinical care of patients [12]. Despite the emergence of advances in technologies has ctDNA reached such a ‘tipping point’ therefore enabling trials for improved outcome directed management of UCB? In other words, has clinical utility been demonstrated? If so, such a validated biomarker is ready for implementation including regulatory approval, commercialization and incorporation into clinical management guidelines [13]. In this editorial, we examine the current state of liquid biopsy in the use of clinical management of UCB from the overview of a recent clinical study published in the May 2019 issue of *Journal of Clinical Oncology*. Conclusions regarding this ‘tipping point’ in terms of current clinical utility of such assays may be obtained from the results.

Earlier studies using liquid biopsy specimens and ctDNA have shown the possibility to identify early metastatic disease detection, disease progression and detect actionable genetic targets in patients with UCB. Most of these studies were proof-of-principle with limited numbers of patients incorporating several types of genetic testing platforms [14–18]. However, more recently Christensen *et al.* have published the results of a comprehensive single-

center, prospective study of 68 patients with clinically localized bladder cancer and longitudinal analysis of ctDNA during well-defined stages of UCB treatment and progression. The study used a personalized tissue-based blood test to track ctDNA at *a priori* defined individual disease timepoints during conventional management of UCB [19].

In this study, all the patients underwent diagnostic transurethral resection for tissue diagnosis at the same time undergoing standard PET/CT staging [19]. Concurrent with this time point blood samples were obtained and DNA was extracted according to a standardized procedure. Subsequently, exome sequencing was performed using libraries of tumor and matching germline DNA to identify mutational signatures previously identified in bladder cancer. Patient-specific somatic variants were selected using plasma multiplex NGS sequencing and matched with clonal mutations after DNA extraction from plasma samples (Signatera™, Natera, Inc.). Sample-level analytic sensitivity has previously been determined to be greater than 95% at a 0.01% ctDNA concentration level.

After initial staging, subjects underwent neoadjuvant chemotherapy using gemcitabine/cisplatin. After appropriate treatment, all patients underwent radical cystectomy with extended lymphadenectomy to the level of the aortic bifurcation (either by open- or robotic-assisted approach). Patients were subsequently followed using CT imaging of thorax and abdomen at regularly scheduled visits. During these visits, plasma specimens were procured from the subjects and genetic expression of ctDNA was analyzed by the aforementioned platform.

The analysis of the detected ctDNA of the subjects over the study period (2013–2017) was shown to have potential utility in terms of clinical meaningful prognosis and disease progression of individuals at several time points of interest. The presence of ctDNA after chemotherapy was associated with pathologic outcomes after subsequent cystectomy. Quite remarkably, 100% of patients with precystectomy detectable ctDNA had residual tumor and/or lymph node metastases. On the contrary, those with ypT0 pathology were ctDNA negative. After NAC and cystectomy, there also was a significant difference in overall recurrence rates between patients with detectable ctDNA and those without (75 vs 0%, respectively, $p < 0.001$). 12-month recurrence rates also showed a similar relationship between the two groups. Moreover, in multivariate Cox proportional hazard regression, ctDNA status after cystectomy was the strongest predictor of recurrence-free survival. The use of longitudinal detection of ctDNA following cystectomy was shown to have a significant lead time over conventional imaging for the detection of clinical recurrence (mean, 96 days). When using strict criteria, serial analysis identified metastatic relapse with 100% sensitivity and 98% specificity. Overall, the results suggest that the presence of ctDNA identifies patients with high-risk disease progression. This biomarker was independent of RNA subtype (basal/squamous, luminal papillary, luminal unstable, stroma-rich), mutational burden and selected gene sets related to chemotherapy response (e.g., *ECCR2* mutations, *DDR* mutations, and immune related gene sets). Moreover, when comparing whole exome sequencing of primary tissue specimens and ctDNA, the investigators found a high degree of similarity in the mutational landscapes between the two suggesting a limited clonal evolution in the disease progression of these selected subjects [19].

While an increasing number of studies show the potential use of ctDNA as a biomarker in applications of cancer management, the feasibility of such management requires foremost the optimization and standardization of preanalytical and analytical methodology. It is within this realm that current gaps in the knowledge may hinder translation to clinical practice [20]. The study by Christensen *et al.* addresses the issues associated with robust sample processing, technical soundness of assays, reproducibility of bioinformatic analysis, the technological and analytical limitations associated with detecting low mutant allele fractions in serum of patients with UCB. In the current study, quality control performed during the workflow showed a 99% sample integrity rate (651 of 656 plasma specimens). While not US FDA approved, the ctDNA platform employed in the study has received 'Breakthrough Device Designation' which offers increased interaction in terms of future regulatory submissions (www.FDA.gov).

Overall, the results of the study suggest that ctDNA may allow guidance in the treatment of patients with UCB. Liquid biopsy based biomarker may find use in risk stratification, improvement in recurrence metastatic detection, assessment of treatment response and prediction of treatment efficacy. Current investigations indicate that longitudinal ctDNA response provides patient-level insight into therapeutic intervention in longitudinal analysis of liquid biopsies of various tumor types, including breast cancer, non-small-cell lung cancer and colorectal cancer [11,21–23]. For non-small-cell lung cancer, ctDNA is used to guide clinical management using *EGFR* mutational analysis. Studies are also underway evaluating the potential assessment of genetic alterations in liquid biopsy for early detection of various cancer types [7,24,25].

What may we conclude regarding the current state in UCB if we reflect on the various stages of cancer biomarker development in the context of developmental stages in other malignancies? In view of the study by Christensen *et al.*, we observe that ctDNA has been defined as a predictive and outcome-directed biomarker with specimen

stability, assay reproducibility and availability. Based on the data discussed herein, it appears that the critical ‘tipping point’ has been met in the development of ctDNA as a potential biomarker in UCB. The next challenges in terms of improving patient outcomes will require investigation in prospective interventional studies to demonstrate significant advantages over existing technology. Meeting such criterion will foster clinical implementation and incorporation into practice guidelines.

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