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Review

Liquid Biopsy-Analysis of Circulating Tumor DNA (ctDNA) in Bladder Cancer

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Abstract. Recent advances in DNA profiling techniques have enabled sensitive detection of tumor-associated genomic aberrations in peripheral blood. This type of minimally-invasive molecular interrogation has the potential to guide subsequent treatment selection. The potential utility of ctDNA in bladder cancer (BC) is bolstered by the high somatic mutation rate, meaning that very small numbers of genes or target regions can be informative. First reports indicate that analysis of ctDNA may represent a sensitive method for disease surveillance in patients with different stages of BC. Moreover, recent evidence suggests that ctDNA analysis reveals previously unknown genomic alterations in metastatic patients. Since some of these gene alterations represent therapeutic targets, ctDNA analysis provides an attractive tool to guide individualized therapy in BC.

Keywords: Biomarker, blood, ctDNA, cfDNA, next generation sequencing, PCR

INTRODUCTION

The analysis of tumor-associated genomic alterations in circulating tumor DNA (ctDNA) is rapidly developing as a platform for biomarker discovery in patients with advanced malignancies [1, 2]. Initial studies of ctDNA in the 1970s described an association between ctDNA burden and tumor aggressiveness [3], but progress was hampered by technological constraints. However, advances in digital droplet polymerase chain reaction (PCR) and highly sensitive next-generation sequencing (NGS) now allow capture and analysis of ctDNA even when highly diluted by non-malignant cell-free DNA (cfDNA) [4, 5]. Recent studies of several solid cancers suggest that somatic alterations detected in ctDNA are reflective of those present in matched tumor tissue: proposing ctDNA-based profiling as a practical method to assess the tumor genome independently of direct tissue analysis [4, 6]. This holds particular promise in patients with relapsed or metastatic disease who rarely undergo biopsy of recurrent or metastatic lesions.

Emerging data suggests that ctDNA analysis will augment the management of patients with bladder cancer (BC) [7–10]. Our review summarizes recent progress and preliminary data supporting clinical utility of ctDNA in BC. In particular, we highlight the remarkable potential for ctDNA to aid disease surveillance across different stages of BC and the power of ctDNA to provide insights into the metastatic BC genome in real-time.

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METHODS

We reviewed current literature nonsystematically using the National Library of Medicine database (http://www.pubmed.gov). A Medline search was performed using the following terms: urothelial carcinoma, bladder cancer, liquid biopsy, cell free DNA, circulating tumor DNA, cfDNA, ctDNA. Articles were considered between 2010 and 2017. References were also secondarily extracted from identified articles. In addition, recent studies presented at the Annual Meeting of the American Society for Clinical Oncology (ASCO) were included.

DETECTION OF CTDNA: METHODOLOGIC CONSIDERATIONS

Fragments of cell-free DNA (cfDNA) are shed into the circulation by both tumor cells and non-malignant cells, especially hematopoietic cells [11]. The enduring challenge for accurate profiling of the tumor-derived proportion of cfDNA, is that the relative quantity of ctDNA varies significantly by disease state and between patients. Unlike a direct tissue biopsy where a pathologist can estimate tumor cellularity prior to genomic profiling, the ctDNA fraction of any given plasma cfDNA sample is much less predictable. Clues can be drawn from a patient's overall disease burden since ctDNA fractions correlate with clinical metrics of tumor volume such as serum alkaline phosphatase or lactate dehydrogenase [12]. In metastatic patients where the ctDNA fraction can be above 50% of all cfDNA, the total extracted DNA yield from plasma can also inform on ctDNA fraction [10]. However, in patients with low disease volume or those with minimal residual disease after therapy, ctDNA may be extremely rare or not be present at all in a small plasma sample. It is important to note that the lower bound for detecting ctDNA is governed by the total amount of cfDNA obtained. In our recent study, the average cfDNA yield in patients with localized muscle-invasive bladder cancer (MIBC) not receiving therapy was less than 10 ng per mL of plasma (representing approximately 1500 diploid genomes) [10]. This means that, even with the latest technologies, for ctDNA to be realistically detected in 1–2 mL of plasma, it must be present at fractions above 1/1500.

In most studies to date, alterations in ctDNA have been detected using either polymerase chain reaction (PCR)-based methods or targeted next-generation sequencing approaches (NGS; Table 1). The selection of the most appropriate method for cfDNA analysis must consider cost, number of genomic alterations and tumor burden. It is not possible to maintain cost-effectiveness while simultaneously informing upon both very low ctDNA fractions and a broad panel of genomic regions. Therefore, approaches are typically tailored for the disease state (and ctDNA fraction) in question. Assays specifically capturing known mutations permit the detection of mutant alleles in patients with ctDNA fractions of 0.01 to 1% [8, 13]. Of course, this must be enabled by either patient-specific a priori knowledge (e.g. from archival tissue profiling) or plausible presence of recurrent single nucleotide mutations (e.g. PIK3CA p.E545K). Droplet digital PCR (ddPCR) or bead-based PCR has been used well to this end, but is challenging to multiplex beyond a handful of mutations [7–9]. Similarly, ultra-deep NGS incorporating unique molecular identifiers (UMIs), also known as barcodes, can reveal very 'diluted' mutations across entire genes, but, due to the high sequencing depth required, costs escalate quickly if scaled beyond a few genes [14]. Importantly however, because of the very high mutation rate in MIBC, both methodologies are suitable and even a very small targeted panel could be effective. For example, over 90% of MIBC patients have at least one mutation in the hotspot regions of PIK3CA, the promoter region of TERT, or the TP53 gene, so a panel covering just those regions can still expect to be informative in most MIBC patients [10, 15, 16].

Broader next-generation sequencing panels allow more exploratory analyses of the genomic landscape, including genes disrupted by copy number changes and rearrangements (such as FGFR3 fusions) [10]. At present this approach is likely only relevant for patients with high ctDNA fractions, such as those with progressing metastatic disease. Indeed, we have shown that whole exome sequencing yields robust results in a significant minority of metastatic BC patients. Unfortunately, with current technology the detection of single gene copy number changes is much more challenging at ctDNA fractions less than 20–30%, due to multiplicative noise [6, 17]. Although shifts in the allelic frequency of germline SNPs can help identify single copy gains or losses at slightly lower ctDNA fractions, we can draw lessons from fetal cfDNA screens of maternal plasma where chromosomal aneuploidies are only confidently 'called' at fetal cfDNA fractions above 10%.

Ultimately the technical challenges of cfDNA analysis must be weighed against those faced by direct

Table 1
Overview of techniques used for analysis of cell free DNA (cfDNA)

Technique	Overview	Minimum % of ctDNA required (of total cfDNA)	Advantages	Disadvantages	Ref.
Digital droplet PCR	Detection of previously known or frequent mutations/CNVs (e.g. androgen receptor in prostate cancer)	~0.01 (mutations) ~10% (copy number alterations*)	High sensitivity, no NGS platform required	Limited number of targets assessable in parallel	[8, 9, 48]
Ultra-deep sequenc-ing with UMIs	Detection of unknown mutations in 1–10 genes or regions	~0.01 (mutations)	High sensitivity, can inform on multiple genes	Either limited to ~1–10 genes with coverage of only few kbps or high costs in case of higher coverage (Mbps)	[14, 27, 49]
Standard targeted NGS (without UMIs)	Detection of unknown mutations/CNVs in a preselected panel of genes or regions	2 (i.e. mutation detection at 1% VAF); 10–20% for CNV detection (varies by nature and extent of alteration)	Broad target regions assessable, low input required	No detection of low abundance mutations	[10, 43, 47]
Standard WES/WGS	Detection of unknown mutations/CNVs	20	Assessment of entire exome/genome	High ctDNA fraction required	[10]

CNV: copy number variation; NGS: next generation sequecing; PCR: polymerase chain reaction; SNP: single nucleotide polymorphism; WES: whole exome sequencing; WGS: whole genome sequencing; VAF = variant allele frequency; UMI = unique molecular identifiers (barcodes); *not yet demonstrated in bladder cancer but shown in other malignancies.

tissue biopsy, which also suffers from variable failure rates, issues of tumor cellularity, and complications derived from formalin-fixation. Blood samples are much more practical to obtain than tissue samples, and blood-based assays are therefore more easily scalable to large patient cohorts. Furthermore, in the event of a negative result (e.g. no ctDNA detected), repeat sampling is clinically feasible.

USING CTDNA FOR BLADDER CANCER SURVEILLANCE

The first reports exploring ctDNA in BC employed ddPCR for highly sensitive detection of distinct BC-associated mutations in plasma, specifically for monitoring patients for relapse in the aftermath of local disease intervention [8, 9]. Birkenkamp-Demtröder et al. demonstrated that low ctDNA fractions in non-muscle-invasive BC (NMIBC) are no barrier to ctDNA detection and clinical utility [8]. In their 2016 report, the authors initially employed WES to identify somatic mutations in primary tumor tissue resected from each patient tumor. Up to six patient-specific mutations from each tumor specimen were then incorporated into a series of ddPCR assays capable of detecting one mutated gene allele from 6000 background copies of the wildtype gene (<0.02%). With this level of sensitivity the authors were able to detect ctDNA early in the disease course in 9 of 12 patients, including patients with Ta disease. Applying these personalized ddPCR assays longitudinally, at multiple time points in the course of each patient's disease, demonstrated ctDNA was detectable prior to clinical demonstration of progression in 4 of 6 patients who ultimately showed clinical progression (it should be noted that all 12 patients with NMIBC in this study developed disease recurrence, and the other 6 progressed to muscle invasive or metastatic disease). Remarkably, in one patient ctDNA was detected 1 year before clinical progression, and assay-specific genome equivalents increased fivefold over this time period. Indeed, average ctDNA burdens were higher in patients who progressed than those who simply recurred. Furthermore, the authors also found high levels of tumor DNA in the urine of all patients with progressive disease, even in the absence of detectable plasma ctDNA. The study strongly supports the use of plasma and urine ctDNA to monitor for BC disease recurrence and progression, and is consistent with recent

data from breast cancer, where digital PCR assays for ctDNA detection can predict relapse after local therapy [4].

Designing personalized ddPCR assays is extremely labour- and cost-intensive (WES of tissue) and requires patient tissue. Given the high mutation rate of some genes in BC, a clear alternative for detecting low ctDNA fractions (and therefore, residual disease) is to develop ddPCR assays targeting a handful of specific genomic alterations with known recurrence. For example, PI3KCA, TERT, FGFR3, RAS family members, and TP53 all contain specific base pairs that are recurrently mutated in BC, known as mutation 'hotspots'. Accordingly, the same Danish group as above applied a non-personalized ddPCR approach in two retrospective patient cohorts. One cohort included 363 patients with NMIBC, and the other included 403 patients undergoing radical cystectomy for predominantly (90%) MIBC. In this study, Christensen et al. selected two of the most commonly mutated genes in BC – *FGFR3* and *PIK3CA* - and developed ddPCR assays for the sensitive detection of their hotspot mutations [18, 19]. However, these two genes alone are not sufficient to represent all patients with BC, and therefore only 36% of NMIBC and 11% of cystectomy patients in this study actually harbored one or both mutations in their archival tumor tissue. Since only 2 of 7 patients in the NMIBC cohort had detectable ctDNA at the time of disease progression, the analysis was not expanded to include all NMIBC patients. In the cystectomy cohort, ctDNA levels were associated with recurrence-free and overall survival. The BC recurred in 8/9 patients with detectable ctDNA but only 6/18 with no detectable ctDNA. The reduced detection rate (particularly for NMIBC), likely reflects the more constrained ddPCR approach, and would likely be improved with the addition of further hotspot mutations in other recurrently mutated BC genes.

The most recent study from the Aarhus group focused on ctDNA to monitor treatment response and disease relapse in patients with MIBC. Predesigned ddPCR assays were used to screen for hotspot mutations in PIK3CA and FGFR3 in tissue samples collected prospectively from 60 patients, of whom 19 were found to have mutations. In addition, the authors performed WES in tumor tissue from 24 of the same patients to develop personalized ddPCR assays. Combined pre-designed and personalized ddPCR assays were used to monitor 26 patients undergoing neoadjuvant chemotherapy followed by radical cystectomy ($n = 24$) or systemic chemotherapy for metastatic BC ($n = 2$). ctDNA was detected in the plasma of 75% of patients after cystectomy, which correlated with worse RFS. The median interval between ctDNA detection in plasma and clinical detection of recurrence was 101 days. A similar early detection of recurrence by ctDNA ahead of radiographic detection has been reported for other cancer types [20]. In ten patients with metastatic disease, the authors observed a clear decrease of ctDNA levels during therapy and an increase after cessation of therapy, which correlated with radiographic response to therapy.

While these three contributions offer proof of principle that plasma ctDNA detected by ddPCR may be a valuable test for disease surveillance, the data is relatively heterogenous, and validation in larger and broader patient cohorts is necessary. As outlined above, the ddPCR approach is attractive due to its sensitivity in patients with low tumor burden, but is limited by the low number of mutations captured, and does not accommodate for tumor heterogeneity and molecular evolution over time.

Further application of this type of assay can be projected for patients with both NMIBC and MIBC. Presence versus absence of ctDNA, or relative quantities of ctDNA could be useful for risk stratification. This could be relevant, for example, in patients with high grade T1 bladder cancer considering intravesical therapy versus immediate radical cystectomy. The administration of adjuvant chemotherapy after radical cystectomy for MIBC could be influenced by the presence of ctDNA in the peripheral blood. Detection of ctDNA could serve as a valuable tool for the early determination of disease recurrence after radical cystectomy or trimodal therapy for MIBC, which in turn would enable administration of early systemic therapy before metastatic disease becomes apparent by radiologic imaging.

A natural extension of using ddPCR to monitor for recurrence and progression in patients with NMIBC and MIBC is to use if for primary disease detection in patients with signs or symptoms of BC but no prior history of BC. Significant efforts are underway to establish protocols using cfDNA for early and non-invasive detection of cancer [21]. It is expected that progress in technical development will significantly reduce costs of current high-throughput approaches, which in turn will promote investigations on ctDNA-based early detection of cancer. In the context of intact primary bladder cancer, analysis of cfDNA in urine

offers an additional option for disease detection and monitoring [4].

Before ctDNA analysis can be implemented in routine clinical practice to monitor the disease status of BC patients, prospective studies with larger sample sizes are required. If studies confirm the potential of ctDNA analysis to detect recurrence earlier than current tools (urine markers, cystoscopy and imaging), interventional studies based on ctDNA detection could be considered. In the case of MIBC, one potential study design would be to select patients for adjuvant chemotherapy based on results of ctDNA analysis early after cystectomy.

MOLECULAR INTERROGATION OF ADVANCED BC USING CTDNA ANALYSIS

Higher plasma ctDNA fractions allow for a more extensive characterization of the corresponding tumor genome using NGS. Since ctDNA burden typically corresponds with disease burden, this type of analysis is largely restricted to advanced disease stages [7]. While The Cancer Genome Atlas Project (TCGA) has provided important insight into the genomics and biology of MIBC, there is surprisingly few data available for metastatic urothelial carcinoma [22, 23]. This is in large part due to the fact that metastatic or recurrent BC is rarely biopsied in clinical practice. Theoretically, analysis of plasma ctDNA enables the molecular characterization of the metastatic tumor ecosystem without the need for invasive biopsy. This may be particularly relevant for BC since a recent study analyzing tissue from multiple metastatic tumor sites of individual patients demonstrated marked heterogeneity between metastases [24]. Furthermore, intra-patient tumor heterogeneity appeared highest in late-stage disease, and would be significantly underestimated using archival primary tissue alone.

A potential advantage of ctDNA analysis is the ability to detect the combined genomic content of all clones from all sites of disease in the context of heterogenous and/or multifocal cancer, provided that each clone releases DNA into circulation. Branched clonal evolution has been described for bladder cancer and other cancers at different tumor sites and under the selective pressure of systemic therapy [24, 25].

A gene panel designed to capture key driver mutations and other actionable alterations in BC, as we have previously reported, will inevitably miss some important genomic alterations. However, our panel captures both truncal and some branch mutations. The addition of more genes to such a panel requires increased breadth of sequencing, invariably increases costs.

Identifying therapeutic targets by next generation sequencing (NGS) of ctDNA

Only two published studies have reported focused data on ctDNA NGS in patients with BC. Patel et al. demonstrated the proof of principle that SNVs can be measured by Tagged Amplicon Sequencing and copy number alterations (CNA) by shallow Whole Genome Sequencing in plasma samples from 17 patients with MIBC receiving neoadjuvant chemotherapy [26]. The assay was designed to analyze SNVs in BRAF, CTNNB1, FGFR3, HRAS, KRAS, NFE2L2, PIK3CA and TP53, which together would capture 72% of patients with MIBC in the original report from the TCGA [22]. Although SNVs and CNAs were found in 12 and 16, respectively, of 16 tumor samples at baseline, only 6 of 17 patients had detectable SNVs or CNAs in their ctDNA before chemotherapy, and 3 additional patients were found to have alterations during chemotherapy. The most frequent alteration was a mutation in TP53. The detection of tumor-associated aberrations in plasma samples before chemotherapy showed no correlation with response to NAC.

We recently reported results from combined whole exome sequencing (WES) and targeted sequencing of 50 genes relevant to BC in plasma cfDNA from 51 patients with aggressive BC, including 37 patients with metastatic disease. Using this broad approach, tumor genomic characterization was limited to patients with ctDNA fractions above 2% (i.e. heterozygous mutations present in plasma at 1% or higher). This mutation detection threshold (1%) is routinely selected in conventional NGS studies because it is approximately 10 times the average background error rate (0.1%) [27]. Accordingly, although only 14% of patients with localized disease had plasma ctDNA fractions above 2%, 73% of patients with untreated metastatic disease exhibited ctDNA above 2% and clear tumor associated genomic changes. In previous studies using different technologic approaches, higher rates of patients with localized tumors and detectable ctDNA have been observed showing that our approach has limitations in non-metastatic disease [16]. Among patients with ctDNA above 2%, the ctDNA fraction relative

to the total cfDNA in the serum ranged from 3.9% to a remarkable 72.6% (i.e. more tumor-derived cfDNA than non-malignant cfDNA in the plasma). ctDNA fractions exceeding 30% were observed exclusively in patients with distant metastatic disease. It seems likely that the majority of ctDNA detected in metastatic patients was shed by metastatic lesions (rather than the primary tumor) as we observed no relationship between cystectomy status and ctDNA fraction. However, we did not assess whether ctDNA burden correlates with metastatic tumor burden (e.g. as assessed by imaging) or number of metastatic lesions.

In patients receiving chemotherapy, we noted that total cfDNA yields from plasma were high regardless of disease stage. Since ctDNA fractions did not appear to be concomitantly elevated, we hypothesize this could reflect increased cfDNA released by dying non-malignant cells affected by chemotherapy. To validate this hypothesis, future studies could explore the spacing of nucleosome footprints in cfDNA to identify putative predominant cell-of-origin. This approach has been shown to enable a characterization of the origin of cells independent of observed genomic alterations [28]. In our study, the TP53 gene was the most commonly altered, affected in 65% (17/26) of patients; higher than the 49% rate observed in the TCGA cohort [22]. Further cell-cycle associated genes with frequent aberrations included RB1 and CDKN2A. A high proportion of patients (19/26) showed aberrations in at least one chromatin remodeling gene (including ARID1A and KMT2D). Alterations in the PI3K/mTOR pathway were observed in a significant proportion of metastatic patients. Genomic alterations in the MAPK pathway were detected in 15/26 patients with evidence of ctDNA, including nine patients with ERBB2 activating changes (either hotspot mutations or gene amplifications). Using capture probes tiled across the introns of FGFR3, we were able to detect (in multiple samples from one patient) a previously unreported gene fusion of FGFR3 and ADD1 potentially leading to overactivation of FGFR3 (similar to the well-established FGFR3-TACC3 fusion gene).

The identification of diverse mutations, copy number changes, and chromosomal rearrangements in our study suggests that ctDNA may be useful for guiding molecularly targeted therapy in advanced BC. For example, detection of FGFR3 fusion genes or ERBB2 amplifications or mutations could identify patients susceptible to FGFR3 or ERBB2 inhibitors, respectively [29]. In clinical trials ERBB2 genomic alterations have been shown to predict sensitivity to a specific HER2 inhibition [30] and FGFR alterations also predict response to FGFR inhibition [31, 32]. Moreover, patients with TSC1/TSC2 alterations may be good candidates for mTOR inhibition (everolimus) [33]. Whether plasma ctDNA analysis can be used as a tool for next generation clinical trials, including especially umbrella or basket trials, is a matter of current debate [34]. First trials using ctDNA analysis in this context have been initiated in various solid malignancies [35].

Markers of response to therapy in ctDNA

Similarly, molecular markers predicting resistance or response to currently approved drugs may be identified in ctDNA. For example, detection of mutations in ERCC2 or other DNA repair genes could be used to identify patients likely to respond to cisplatin-based chemotherapy [36–39]. This type of genomic prediction could streamline care to avoid ineffective and expensive treatments and instead prioritize the most effective therapies.

Since high mutational load in BC tumor tissue is proposed as a potential biomarker for patient response to immune checkpoint blockade, we have suggested that assessment of the mutation rate using plasma ctDNA should be tested for its ability to predict response [40, 41]. In our cohort, 12% of patients had projected mutation rates above 30 per MB, a threshold that has been identified in the tissue of other cancers to be associated with response to immunotherapy. In a subgroup of patients, we performed WES and compared mutation rates derived from WES with rates derived from targeted sequencing. Although higher mutation rates were measured by targeted sequencing compared to WES, estimated somatic mutation rates correlated significantly between both methods. The higher mutation rate in targeted sequencing may result from a bias towards recurrent driver mutations in the targeted gene panel, as well as the ability of deep targeted sequencing to detect low allele frequency sub-clonal mutations that are missed by conventional WES. This observation highlights the need to determine thresholds for ‘high’ mutational load that are specific to the assay in question. Furthermore, the specific type of mutation, not just overall mutation rate, may be important. A recent analysis of 5777 solid tumours from 19 cancer types suggested that insertion or deletion (indel) mutations may be associated with higher neoantigen immunogenicity

compared to non-synonymous single nucleotide variants (SNVs). In a subset of 38 melanoma patients, higher counts of frameshift indel mutations were significantly associated with response to PD-1 inhibition [42]. High rates of indels in kidney tumors may also help explain the responses observed to immune checkpoint blockade in that cancer type, despite an overall low mutation rate on average. Future studies must address these critical knowledge gaps in BC.

The analysis of plasma samples collected in the framework of currently ongoing phase III trials using immunotherapy in urothelial carcinoma will hopefully provide more insight into a potential correlation between neoantigen burden and response to immunotherapy. If such a correlation can be confirmed, prospective interventional trials selecting treatment type (chemotherapy or immunotherapy) based on liquid biopsy profiles could be considered.

In a recent study presented at ASCO 2017, Kuziora et al. analyzed plasma ctDNA using a targeted sequencing panel of 70 genes in 29 patients with metastatic urothelial carcinoma before treatment and after 6 weeks of treatment with the PD-L1 inhibitor durvalumab in the framework of a phase II trial [43]. In accordance with our own data, the genes most frequently altered in pre-treatment samples were TP53 (73%) and ARID1A (55%). The authors did not assess mutational burden at baseline as a predictor of response, as we propose above, but instead investigated whether changes in variant allele frequency (VAF) on treatment correlated with response. Compared to baseline, patients with response to durvalumab showed a significant decrease in the variant allele frequency after 6 weeks of treatments (–2.36%, $p = 0.02$), whereas no significant changes in VAF were detected in patients with progressive disease (+2.69%, $p = 0.31$). Moreover, patients with response to durvalumab had a decreased mutation count after 6 weeks of treatment (–4.6 counts, $p = 0.003$) whereas no significant change was detected in patients with progressive disease (+2.78 counts, $p = 0.436$). Patients showing a decrease in the VAF after 6 weeks had a significantly better PFS and OS. These data suggest that changes in cfDNA profiles detected during an early phase of treatment may serve as a surrogate parameter of response to treatment and improved outcome. Future studies should examine whether lack of ctDNA decline on treatment can be used as a tool to identify patients who will not respond to a given therapy, and therefore facilitate an earlier change in therapy

ctDNA in the context of tumor heterogeneity

It is not yet possible to determine whether genomic aberrations present in the plasma ctDNA originate from multiple lesions or from a single progressing lesion. Molecular heterogeneity is as much of a concern in BC as it is in most cancers, such that a putative target or marker of drug response may only be present in part of a tumor or in one tumor site [44, 45]. As described above, the seminal study from Faltas and colleagues described remarkable intra-patient tumor heterogeneity in BC [24]. In theory ctDNA could reflect the totality of all tumor sites, or at least the predominant tumor clone. Longitudinal testing over time would allow emerging clones to be identified promptly. To assess how multiple lesions affect the profile of ctDNA in patients with metastatic BC, future studies comparing multiple metastatic sites and ctDNA will be needed. A rapid autopsy program could provide a suitable setting for this type of study [46].

In this context Cheng et al. reported at ASCO 2017 on correlations between ctDNA analysis and

Table 2
Potential applications of ctDNA analysis in patients with different bladder cancer (BC) disease states

Disease State	Potential Application of ctDNA	References
NMIBC	• Screening/Diagnosis	[8]
	• Risk stratification: Prediction of recurrence/progression	[9]
	• Prediction of response to intravesical therapy	
	• Monitoring for recurrence	
MIBC	• Risk stratification: Prediction of recurrence/survival	[26]
	• Prediction of response to neoadjuvant chemotherapy	[10]
	• Decision aide for adjuvant chemotherapy for clinically occult disease (+ctDNA)	
	• Monitoring for recurrence	
Metastatic BC	• Determination of mutational load (as putative marker of response to immunotherapy)	[10]
	• Liquid biopsy for molecular characterization	[47]
	• Determine presence of target for targeted therapy	[43]
	• Prediction of response to therapy	

profiling of archival tissue in 15 patients with metastatic urothelial carcinoma using a capture-based NGS assay targeting 341–468 genes [47]. In the whole cohort of patients ($n = 26$, including patients with missing archival tissue), at least one somatic mutation was detected in ctDNA of 69% of patients, but the same alterations were not present in the tumor tissue in 40% of cases. On the other hand, 73% of patients exhibited alterations in archival tissue that were not detected in plasma. An identical mutation profile in tissue and plasma was detected in 20% of patients. The interval between tumor tissue sampling and plasma sampling in these patients was between 35 days and 1.5 years. These findings underline the potential added value of plasma ctDNA analysis compared to sequencing of archival tumor tissue, which might not reflect the current status of disease.

SUMMARY

Bladder cancer exhibits a high mutational burden compared to other solid tumors. This characteristic means that bladder cancer is particularly suited for ctDNA analysis, as 'diluted' mutant alleles can now be robustly detected in peripheral blood using state-of-the-art technologies. In the context of disease surveillance, initial studies have shown that specific ddPCR assays facilitate early and highly sensitive detection of disease recurrence. In patients with metastatic BC, early targeted NGS studies have demonstrated that the vast majority of progressing patients have high levels of ctDNA in their blood and harbor genomic aberrations that may associate with differential sensitivity to specific drugs. In addition to its potential utility in surveillance and identification of treatment-relevant genes, ctDNA may also be a valuable tool for detection of markers of response to therapy and other components of patient management (Table 2). This is a burgeoning field of study with many new discoveries likely in the near future.

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CONFLICTS OF INTEREST

No relevant conflicts of interest.

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