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Seminars Article

IBCN Seminar Series 2021: Circulating tumor DNA in bladder cancer

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1. Introduction

In this issue of the IBCN Seminar Series, we present and discuss the properties of circulating tumor DNA (ctDNA) and the potential implications of ctDNA-based research in the management of patients with bladder cancer. We furthermore summarize encouraging results from the field and ongoing and future clinical trials utilizing ctDNA.

2. Biology and detection of ctDNA

Cell-free DNA (cfDNA) is continually shed into the circulation from dying cells. cfDNA is highly fragmented and present at fragment sizes of approx. 166 base pairs corresponding to the stretches of DNA protected by the nucleosome [1,2]. Tumors also release cfDNA, which harbors tumor-specific (somatic) alterations and is termed ctDNA [3]. cfDNA is thought to primarily be released from apoptotic or necrotic cells, however active release mechanisms have also been described [4]. Importantly, the half-life of cfDNA has been estimated to be below 2 hours, which makes cfDNA capable of providing real-time monitoring of the tumor burden [5]. ctDNA detection is more frequent with higher tumor burden and with increasing tumor invasiveness [6,7]. As a product of tumor cells and a reflection of the tumor burden, ctDNA inherently has the potential to be used for screening, early diagnosis, prognosis, minimal residual disease detection, treatment response monitoring and evaluation of clonal evolution [8].

The detection of ctDNA can be based on either a tumor-informed or a tumor-agnostic approach. Tumor-agnostic describes a scenario in which the somatic genomic features of the tumor are unknown prior to cfDNA testing, and generally requires broad targeted sequencing panels to discover these somatic genomic features. In contrast, a tumor-informed approach means that key somatic genomic features are already known, typically from genomic profiling of tumor tissue. The tumor-informed approach has been utilized extensively for the purposes of ctDNA detection due to increased sensitivity [7]. Tumor-agnostic approaches are more commonly used for de novo characterization of targets for treatment and resistance-conferring genomic

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alterations [2]. Targeted sequencing methods have been widely used to detect ctDNA, but are limited by the available input material as DNA fragments with tumor-specific mutations in the targeted regions might not be present in the analyzed sample. Whole genome sequencing is now being employed in a tumor-informed fashion to enable characterization of vastly more mutations, which translates into fewer DNA fragments needing to be analyzed to capture a ctDNA-derived signal [9].

3. Prognostic and predictive value of ctDNA detection in localized bladder cancer

The prognostic value of ctDNA was demonstrated in a recent study on plasma ctDNA including 68 patients with muscle-invasive bladder cancer (MIBC) treated with neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC). The authors reported associations between patient outcome and ctDNA status determined at both diagnosis, before RC and after RC (multiple time points investigated) [10]. The vast majority of patients with detection of ctDNA after RC developed BC recurrence and, interestingly, most of these patients were ctDNA-positive already at diagnosis. Importantly, the detection of ctDNA after RC preceded radiological detection of BC recurrence in most patients with a median lead-time of approx. 3 months. In the same study, the dynamics of ctDNA during treatment, that is clearance or persistence of ctDNA, were associated with response to NAC and hence demonstrated the predictive nature. However, a group of patients demonstrated ctDNA clearance, but lack of response to NAC. Interestingly, when compared to recurrence following RC, ctDNA dynamics showed a superior association compared to pathologic response to NAC.

In the IMvigor010 adjuvant trial, a trial that did not meet the primary endpoint, a sub analysis of patients positive for ctDNA after RC had shorter disease-free and overall survival compared to those negative for ctDNA (possible prognostic role). Moreover, patients positive for ctDNA after RC had longer disease-free and overall survival when treated with adjuvant atezolizumab compared to observation [11]. No significant difference in survival was identified for patients negative for ctDNA, suggesting that ctDNA status could possibly be a predictive biomarker for benefit with atezolizumab in the adjuvant setting. Clearance of ctDNA during treatment was also found to be associated with improved survival (possible predictive role). In this study, ctDNA analyses were not performed at multiple timepoints during treatment, which may limit dynamic analyses in the context of tumor response,

4. ctDNA in the metastatic setting

Studies have shown high levels of ctDNA to be present in patients with clinically progressing metastatic disease, with a median ctDNA fraction of approx. 10%. However, plasma ctDNA levels vary widely between patients and a proportion may have levels below 1% [12]. Importantly, even in the metastatic setting, the level of ctDNA seems clinically prognostic and positively associated with aggressive disease features [13]. The level of ctDNA is particularly important for identification of targets for treatment in the metastatic setting, as for example, copy number changes require relatively high levels of ctDNA to enable robust detection compared to other types of genomic alterations, such as single nucleotide variants [14].

High concordance has been observed between the mutational landscape of patient-matched plasma samples and tumor tissue; however, concordance is less clear in plasma samples with low ctDNA levels [15]. As an extension hereof, the mutational landscape in metastatic disease, based on ctDNA analysis, shows great similarity to the mutational landscape of MIBC defined by primary tumor analysis [13]. Clinically-relevant genomic alterations can be identified in ctDNA, suggesting opportunities for precision oncology [16,17]. For example, in patients with *FGFR3* or *ERBB2 (HER2)* gene alterations identified in ctDNA, the corresponding mRNA and proteins are significantly upregulated in tumor tissue [13]. Therefore it is plausible that ctDNA testing could help identify tumors that are potentially sensitive to therapies targeting FGFR, HER2, or other potentially “actionable alterations”; such hypotheses can be further tested in clinical trials.

5. Other examples of potential utility

The use of ctDNA has been suggested as an approach for cancer screening, provided that the presence of ctDNA entails the presence of tumor cells [18]. This does, however, necessitate a tumor-agnostic approach or an approach that enriches mutational sites that are often altered. Importantly, as the level of ctDNA is a reflection of the tumor burden, a screening setting requires a very sensitive method. To address this, it could be feasible to enrich the screening population based on high risk factors in future setups. Importantly, novel results from a multi-cancer early detection test have shown limited sensitivity for detection of stage I cancers (sensitivity 16.8%) [19].

Plasma ctDNA may also be useful for determining tumor mutational burden (TMB), since same-patient ctDNA-based TMB and tissue-based TMB are highly correlated in metastatic urothelial carcinoma [13]. In addition, a recent ctDNA-focused pan-cancer study of approx. 10,000 patients demonstrated an association between ctDNA-based TMB and patient outcomes [20]. The ongoing PREVAIL study including patients with advanced urothelial carcinoma at the first line treatment setting may further evaluate this question. ctDNA has been detected in urine supernatants in patients with MIBC. The mutated DNA fragments identified in such samples might represent lysis of tumor cells within the bladder lumen or clearance of plasma ctDNA, as suggested by identification of high levels of urine ctDNA in

patients after completion of RC [21]. Importantly, high levels of ctDNA in urine supernatants obtained before RC have also been associated with lack of response to NAC and poor outcome following RC [22,23]. In line with this, a recent study demonstrated persistence of ctDNA in urine supernatants during NAC treatment to be associated with response to treatment [24].

6. ctDNA in clinical trials

Multiple studies have demonstrated promising results for ctDNA analyses with the potential to influence clinical practice in the management of patients with MIBC. The next vital step is to demonstrate improved patient outcomes, quality of life and hopefully reduced cost associated with the treatment regimens through clinical trials. The study by Christensen et al. suggested multiple approaches for clinical trial designs [10]. Initially, at diagnosis of MIBC, lack of ctDNA detection has been associated with better prognosis and patients without detectable ctDNA might potentially be spared NAC. Patients with detectable ctDNA appear to be at elevated risk and should receive NAC with monitoring of ctDNA during treatment. In the event of ctDNA clearance, suggesting sensitivity to chemotherapy, additional cycles might be offered, whereas if ctDNA remains detectable it might be favorable to change treatment regimen or move to early RC if no alternative systemic options exist. Detection of ctDNA after RC has been demonstrated as an indication of metastatic disease and could serve as the deciding factor for initiating treatment regardless of detection of metastatic disease by imaging. In patients undergoing treatment of metastatic disease, monitoring ctDNA during treatment could provide a real-time indication of treatment response and serve as evidence for changing treatment regimen if ctDNA persists. All the above hypotheses need to be tested in prospective clinical trials before incorporation into clinical practice.

The ongoing TOMBOLA trial (NCT04138628) addresses the potential for initiating treatment earlier based on ctDNA detection. It is a single-arm, non-randomized phase II trial with an estimated enrollment of 282 patients in order to identify 127 ctDNA-positive patients and initiate treatment with atezolizumab. The primary endpoint on the trial is response measured by a combination of ctDNA status and CT scan results. Imvigor011 (NCT04660344) is an ongoing trial that builds on the seminal findings of the Imvigor010 trial [11]. It is a randomized, placebo-controlled phase III trial that addresses the efficacy and safety of treatment with atezolizumab (vs. placebo) only in patients who are ctDNA-positive after RC. It has an estimated enrollment of 495 patients in order to randomize 213 patients with detectable ctDNA after RC. The primary endpoint in the trial is disease free survival. Both the TOMBOLA and Imvigor011 trials thereby address the potential for survival benefit based on initiating treatment at an earlier time point based on detection of ctDNA compared to waiting for disease detection by imaging according to current standards of care. This step of prospective assessment of clinical utility is critical before routine use of ctDNA in clinical practice.

In addition, efforts are underway to establish an umbrella trial for initiating targeted treatments based on findings from broader characterizations of ctDNA in patients with locally advanced or metastatic bladder cancer, similar to a current trial for patients with metastatic castration-resistant prostate cancer (NCT03385655).

7. Discussion

Despite the recent advances in research on ctDNA and progress in clinical trials, there remain a number of elements regarding the biology and clinical implications of ctDNA that we still do not fully understand. Theoretically, we expect most tumor lesions in a patient to shed ctDNA, although to various extents, depending on the invasiveness and aggressiveness of the lesion [25]. Limited evidence is currently available that addresses this, however a recent study on ctDNA from patients with non-small cell lung cancer (NSCLC) demonstrated detection of mutations private to specific metastatic lesions in the plasma of a single extensively analyzed patient [6]. Notably, a number of tumor subclones originating from metastatic lesions were not detected, demonstrating that there might be instances with no or at least not detectable ctDNA from a number of lesions. Consequently, it remains unclear to what extent the genomic landscape observed by ctDNA analysis reflects the heterogeneity and genomic landscape of all metastatic disease and, furthermore, how this landscape evolves under the selective pressure applied by treatments over time. This could also have implications for evaluation of resistance mechanisms, future treatment guidance and therefore requires further research to fully understand.

Another key aspect of ctDNA analysis is the biology behind ctDNA shedding. Studies consistently report a lack of ctDNA detection in a number of patients with significant tumor burden and poor outcome, where the detection of ctDNA would have been expected. This might be attributable to insufficient sensitivity of the applied methods, but might also reflect biologic characteristics resulting in no or very limited shedding of ctDNA. By performing gene expression analysis of primary tumor tissue from TURBT, Powles et al. found higher expression of cell cycle and keratin genes in patients shedding ctDNA and the study by Abbosh et al. on ctDNA in NSCLC demonstrated a high Ki-67 proliferation index, lymphovascular invasion and non-adenocarcinoma histology to be associated with shedding of ctDNA [6].

The timing of sample draw is another important aspect for application of ctDNA analysis in detection of residual disease after surgery. Recent data have shown that the level of cfDNA is particularly elevated up to 4 weeks following surgery, which could necessitate interrogation of additional

cfDNA molecules for identification of ctDNA and thereby affect sensitivity [26]. Furthermore, the sensitivity for ctDNA detection would be expected to vary between frameworks employing either longitudinal sampling or a sample from a single time point. This scenario might to some extent be reflected in the different recurrence rates observed for patients who are ctDNA negative following RC in the publications by Christensen et al. and Powles et al. at 0% and 30.6% (observation arm), respectively [10,11]. Both studies employ the Signatera analytical framework for ctDNA detection, but the former analyzes longitudinal samples and the latter samples obtained at the time of treatment initiation. However, sample handling and processing differences between the studies might explain part of the observed difference.

8. Conclusion

ctDNA analysis represents a highly promising avenue for supplementing the current management of patients with MIBC in the future. Data suggest that it might potentially serve as a powerful prognostic biomarker at the time of BC diagnosis, during treatment with NAC and before RC. In addition, it seems to be associated with recurrence risk after RC with an estimated 3-month lead-time compared to imaging techniques. In the adjuvant setting, data suggest a putative predictive role of ctDNA analysis for treatment with immune checkpoint inhibitor, which requires prospective validation. Furthermore, tracking response to treatment is a promising feature of ctDNA analysis. Importantly, the most promising results obtained in the setting after RC are currently being put to the test in prospective clinical trials, for example, IMvigor011 and TOMBOLA. It will be important for future clinical implementation to demonstrate that overall survival and quality of life have been improved. Furthermore, in the NMIBC setting it is possible that urine ctDNA may also help identify or monitor disease recurrence, although this is not yet well-explored. However, many unanswered questions remain about ctDNA that pertain to its biology, shedding, the optimal time points for sampling and its capability to be representative of the full genomic landscape of the totality of metastatic disease, including copy number changes. Resolution of these questions through ongoing research could add significantly to the very promising data for ctDNA and its potential role in the future as a critical component in the clinical practice for patients with MIBC.

Conflicts of interest

The authors declare no conflicts of interest relating to this manuscript, as per the seminar article structure. The manuscript is an extended summary of an online seminar and therefore reflects presentations of already published work and details of already established clinical trials.

References

[1] Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. Cell 2016;164:57–68.
[2] Newman AM, Bratman SV, To J, Wynne JF, Eclov NCW, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548–54.
[3] Leary RJ, Sausen M, Kinde I, Papadopoulos N, Carpten JD, Craig D, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 2012;4:162ra154.
[4] Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. Clin Chim Acta 2001;313:139–42.
[5] Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985–90.
[6] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545:446–51.
[7] Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
[8] Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223–38.
[9] Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. Nat Med 2020;26:1114–24.
[10] Christensen E, Birkenkamp-Demtröder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. J Clin Oncol 2019;37:1547–57.
[11] Powles T, Assaf ZJ, Davarpanah N, Banchereau R, Szabados BE, Yuen KC, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nature 2021;595:432–7.
[12] Vandekerkhove G, Todenhöfer T, Annala M, Struss WJ, Wong A, Beja K, et al. Circulating tumor DNA reveals clinically actionable somatic genome of metastatic bladder cancer. Clin Cancer Res 2017;23:6487–97.
[13] Vandekerkhove G, Lavoie J-M, Annala M, Murtha AJ, Sundahl N, Walz S, et al. Plasma ctDNA is a tumor tissue surrogate and enables clinical-genomic stratification of metastatic bladder cancer. Nat Commun 2021;12:184.
[14] Herberts C, Wyatt AW. Technical and biological constraints on ctDNA-based genotyping. Trends Cancer Res 2021;7:995–1009.
[15] Sundahl N, Vandekerkhove G, Decaestecker K, Meireson A, De Visschere P, Fonteyne V, et al. Randomized phase 1 trial of Pembrolizumab with sequential versus concomitant stereotactic body radiotherapy in metastatic urothelial carcinoma. Eur Urol 2019;75:707–11.
[16] Grivas P, Kiedrowski LA, Sonpavde GP, Gupta SV, Thomas RA, Gourdin TS, et al. Spectrum of FGFR2/3 alterations in cell-free DNA of patients with advanced urothelial carcinoma. Bladder Cancer 2021:1–6.
[17] Grivas P, Lalani A-KA, Pond GR, Nagy RJ, Faltas B, Agarwal N, et al. Circulating tumor DNA alterations in advanced urothelial carcinoma and association with clinical outcomes: a pilot study. Eur Urol Oncol 2020;3:695–9.
[18] Gormally E, Vineis P, Matullo G, Veglia F, Caboux E, Le Roux E, et al. TP53 and KRAS2 mutations in plasma DNA of healthy subjects and subsequent cancer occurrence: a prospective study. Cancer Res 2006;66:6871–6.

[19] Klein EA, Richards D, Cohn A, Tummala M, Lapham R, Cosgrove D, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol 2021;32:1167–77.

[20] Zhang Y, Yao Y, Xu Y, Li L, Gong Y, Zhang K, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. Nat Commun 2021;12:11.

[21] Birkenkamp-Demtröder K, Christensen E, Nordentoft I, Knudsen M, Taber A, Høyer S, et al. Monitoring treatment response and metastatic relapse in advanced bladder cancer by liquid biopsy analysis. Eur Urol 2018;73:535–40.

[22] Christensen E, Birkenkamp-Demtröder K, Nordentoft I, Høyer S, van der Keur K, van Kessel K, et al. Liquid biopsy analysis of FGFR3 and PIK3CA hotspot mutations for disease surveillance in bladder cancer. Eur Urol 2017;71:961–9.

[23] Chauhan PS, Chen K, Babbra RK, Feng W, Pejovic N, Nallicheri A, et al. Urine tumor DNA detection of minimal residual disease in muscle-invasive bladder cancer treated with curative-intent radical cystectomy: a cohort study. PLoS Med 2021;18:e1003732.

[24] Patel KM, van der Vos KE, Smith CG, Mouliere F, Tsui D, Morris J, et al. Association of plasma and urinary mutant DNA with clinical outcomes in muscle invasive bladder cancer. Sci Rep 2017;7:5554.

[25] Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC — challenges to implementing ctDNA-based screening and MRD detection. Nat Rev Clin Oncol 2018;15:577–86.

[26] Henriksen TV, Reinert T, Christensen E, Sethi H, Birkenkamp-Demtröder K, Gögenur M, et al. The effect of surgical trauma on circulating free DNA levels in cancer patients-implications for studies of circulating tumor DNA. Mol Oncol 2020;14:1670–9.