Clinical and Translational Radiation Oncology 43 (2023) 100682

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.sciencedirect.com/journal/clinical-and-translational-radiation-oncology

Case Report

Early circulating tumor DNA dynamics at the commencement of curative-intent radiotherapy or chemoradiotherapy for NSCLC

Michael MacManus [a,b], Laura Kirby [a], Benjamin Blyth [a,b], Owen Banks [a], Olga A. Martin [a,b,c], Miriam M. Yeung [a,b], Nikki Plumridge [a], Mark Shaw [a], Fiona Hegi-Johnson [a,b], Shankar Siva [a,b], David Ball [a,b], Stephen Q. Wong [a,b,1,*]

[a] Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
[b] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC, Australia
[c] Centre for Medical Radiation Physics, University of Wollongong, Wollongong, New South Wales, Australia

ARTICLE INFO

Keywords:
Circulating tumor DNA
Radiotherapy
Chemoradiotherapy

ABSTRACT

Background: The kinetics of circulating tumor DNA (ctDNA) release following commencement of radiotherapy or chemoradiotherapy may reflect early tumour cell killing. We hypothesised that an increase in ctDNA may be observed after the first fraction of radiotherapy and that this could have clinical significance.
Materials and methods: ctDNA analysis was performed as part of a prospective, observational clinical biomarker study of non-small cell lung cancer (NSCLC) patients, treated with curative-intent radiotherapy or chemoradiotherapy. Blood was collected at predefined intervals before, during (including 24 h after fraction 1 of radiotherapy) and after radiotherapy/chemoradiotherapy. Mutation-specific droplet digital PCR assays used to track ctDNA levels during and after treatment.
Results: Sequential ctDNA results are available for 14 patients with known tumor-based mutations, including in *EGFR*, *KRAS* and *TP53*, with a median follow-up of 723 days (range 152 to 1110). Treatments delivered were fractionated radiotherapy/chemoradiotherapy, in 2–2.75 Gy fractions (n = 12), or stereotactic ablative body radiotherapy (SABR, n = 2). An increase in ctDNA was observed after fraction 1 in 3/12 patients treated with fractionated radiotherapy with a complete set of results, including in 2 cases where ctDNA was initially undetectable. Neither SABR patient had detectable ctDNA immediately before or after radiotherapy, but one of these later relapsed systemically with a high detected ctDNA concentration.
Conclusions: A rapid increase in ctDNA levels was observed after one fraction of fractionated radiotherapy in three cases. Further molecular characterization will be required to understand if a "spike" in ctDNA levels could represent rapid initial tumor cell destruction and could have clinical value as a surrogate for early treatment response and/or as a means of enriching ctDNA for mutational profiling.

Introduction

The primary curative-intent treatment modality for patients with unresectable non-small-cell lung cancer (NSCLC) is radiotherapy (RT), either given alone or with concurrent platinum-based chemotherapy [1]. Small, favorably located tumors can be treated with stereotactic body ablative radiotherapy (SABR). Patients with unresectable, stage III NSCLC may receive adjuvant anti-PD-L1 antibody durvalumab after chemoradiation [2]. The use of immunotherapy has been a major advance in NSCLC. Early cell death may be immunogenic and could have significance in the immunotherapy setting. Despite therapeutic advances, most patients with locoregionally advanced NSCLC die with recurrent disease. Circulating biomarkers that could predict or accurately-assess therapeutic responses and guide subsequent treatment management decisions would be extremely valuable.

The analysis of circulating tumor DNA (ctDNA) provides enormous

Abbreviations: ctDNA, circulating tumor DNA; NSCLC, Non-small cell lung cancer; SABR, stereotactic ablative body radiotherapy; RT, radiotherapy; FDG-PET, ^{18}F-labeled fluorodeoxyglucose positron emission tomography; ddPCR, droplet digital PCR.

* Corresponding author at: Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia.
E-mail address: stephen.wong@petermac.org (S.Q. Wong).
[1] Postal address: Locked Bag 1, A'Beckett Street, Melbourne Vic 8006 Australia.

https://doi.org/10.1016/j.ctro.2023.100682
Received 22 August 2023; Accepted 21 September 2023
Available online 22 September 2023

Table 1
Baseline characteristics of patients.

Patient Characteristics	Number of Patients (%)
Age, y, median (range)	69.5 (56-80)
Gender	
M	6 (43.9%)
F	8 (57.1%)
Stage	
I	2(14.3%)
II	2(14.3%)
III	8(57.1%)
IV	2(14.3%)
Mutation identified from tumor	
KRAS	8 (57.1%)
EGFR	3(21.4%)
TP53	3 (21.4%)
Treatment	
SABR	2 (14.3%)
RT	5 (35.7%)
Chemo/RT	4 (28.6%)
Chemo/RT + durvalumab	3 (21.4%)

Y, year; M, Male; F, Female; SABR, stereotactic ablative body radiotherapy; RT, radiotherapy, Chemo/RT, Chemoradiotherapy.

potential as a non-invasive prognostic and predictive biomarker that can be used to track disease kinetics following treatment [3]. However, the clinical validity of using ctDNA in the context of radiotherapy or chemoradiotherapy treatment is not well understood. Importantly, since both treatment modalities result in acute tumor cell death, we hypothesized that ctDNA levels may rapidly rise after the first fraction of radiotherapy exposure and provide a readout of a responsive tumor. Through the analysis of plasma samples collected before and shortly after initial treatment from a cohort of NSCLC patients, this study aimed to determine if this rapid ctDNA rise is observed.

Materials and methods

Patients

Samples were derived from a prospective, observational clinical study of NSCLC patients treated with curative-intent radiotherapy, with or without concurrent chemotherapy (Australian New Zealand Clinical Trials Registry ACTRN1262200079173). Written informed consent was obtained from all patients under an approved Human Research Ethics Committee protocol. Fractionated RT was 60 Gy in 30 fractions with concurrent chemotherapy or 55 Gy in 20 fractions single-modality.

Study conduct

Blood was collected at predefined intervals prior to treatment, 24 h after the first fraction of radiotherapy or chemoradiation, during treatment, post-treatment (4–6 weeks after treatment cessation) and at the time of disease progression. Patients had baseline and post treatment ^{18}F-labeled fluorodeoxyglucose positron emission tomography (FDG-PET) scans and were followed up for clinical outcomes including progression-free and overall survival. Post-treatment blood collection coincided with FDG-PET.

Circulating tumor DNA analysis

Tumour specific mutations were identified through routine molecular diagnostic testing of tumor tissue. Up to 5mls of plasma were extracted for cell-free DNA using the Qiagen circulating nucleic acid extraction kit. Cell-free DNA was isolated from up to 5 ml of plasma and droplet digital dPCR (ddPCR) was performed using the Bio-Rad QX200 ddPCR system. ctDNA was defined as detectable if there was ≥ 1 copy of mutant DNA detected in both duplicate reactions.

Results

Between December 2016 and January 2022, 74 patients with diagnosed stage I-IV NSCLC treated with radiotherapy were enrolled. To facilitate ctDNA analysis via a tumor informed approach, key somatic driver mutations were identified from routine molecular testing results in 14 patients in genes such as *KRAS* (n = 8), *EGFR* (n = 3) and *TP53* (n = 3) (Table 1, Supplementary Table S1). Overall, there were 2 stage I, 2 stage II, 8 stage III (n = 12) and 2 oligometastatic stage IV patients. Fractionated RT for stage II-IV was given as single modality (n = 5), concurrent chemoRT (n = 4) or chemoRT followed by adjuvant durvalumab (n = 3). Both stage I patients received SABR. The median follow-up was 723 days (range 152 to 1110).

ctDNA analysis was performed in a total of 73 plasma samples collected from the 14 patients using mutation specific digital PCR assays with all patients analysed at a baseline timepoint prior to treatment, at a 24-hour timepoint after the first fraction of radiotherapy and at least two timepoints following treatment including at the time of disease progression (Median total of 5 timepoints per patient). At baseline, ctDNA was detected in 9/14 patients with a median ctDNA level of 16 copies/ml of plasma (range 0–2880 copies/ml) and a median variant allele frequency (VAF) of 0.18 % (range 0–10.2 %). Consistent with other reports [4], ctDNA levels provided prognostic information with detection of ctDNA associated with worse progression free and overall survival, although this did not meet statistical significance due to the limited number of cases analysed (Supplementary Fig. S1).

Serial analysis of ctDNA levels closely mirrored the clinical course of patients as assessed by PET imaging. For example, in patients with either a partial or complete metabolic response to treatment (LCB002, LCB0065, LCB0068), ctDNA levels decreased from baseline and remained undetectable for several weeks following treatment (Fig. 1A). In contrast, multiple patients had increasing levels of ctDNA following treatment that coincided with progressive disease as assessed by PET imaging (LCB005, LCB049, LCB051) (Fig. 1B). In general, these results reaffirm the potential clinical utility of ctDNA analysis to provide a prognostic and disease monitoring tool for NSCLC cancer patients.

To understand how radiotherapy affects early ctDNA dynamics, we examined ctDNA levels from a plasma sample collected 24 h immediately following treatment. ctDNA was detected in 10/14 patients at this timepoint. We defined those with an increase or decrease in ctDNA levels based on a greater than 30 % difference in ctDNA levels between the baseline and the 24 h timepoint (Supplementary Table S1).

In 3/14 (21.4 %) cases, there was no detection of ctDNA at baseline, including both stage I patients treated with SABR, suggesting disease burden was below the threshold of detection. Neither SABR patient had a detectable spike in ctDNA but one had a subsequent systemic relapse, associated with high detected ctDNA levels, confirming the ability of the assay to detect above threshold for ctDNA detection in that case (Fig. 2). In 5/14 patients (35.7 %), ctDNA levels decreased after fraction 1 and 3/14 (21.4 %) patients had no significant change in ctDNA between the two timepoints. Significantly, we observed an increase in ctDNA in 3 patients, including in 2 cases where baseline ctDNA was undetectable (Fig. 3). All 3 patients had histologically proven adenocarcinomas with a *KRAS* activating mutation. Two of these patients had stable disease following treatment and one patient had a partial metabolic response. Overall, in this study, ctDNA levels exhibited widely differing early

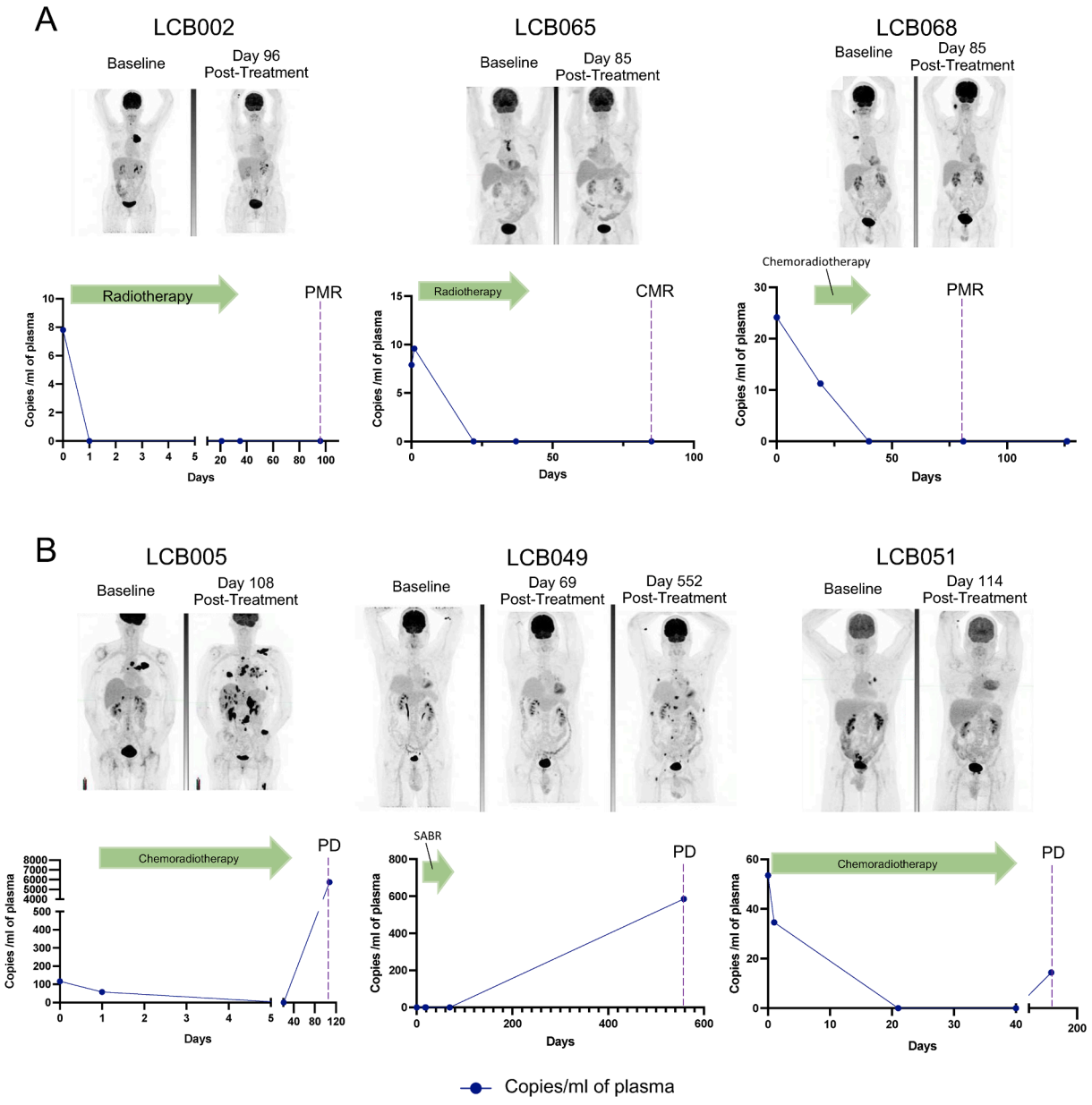

Fig. 1. Longitudinal analysis of ctDNA levels in patients with A) either a partial or complete metabolic response to treatment or with B) progressive disease following initial treatment. Absolute levels of ctDNA are shown for each patient with response to treatment as assessed by FDG-PET imaging indicated by the dashed line. CMR, Complete metabolic response; PMR, Partial metabolic response; PD, Progressive disease, SABR, stereotactic ablative body radiotherapy.

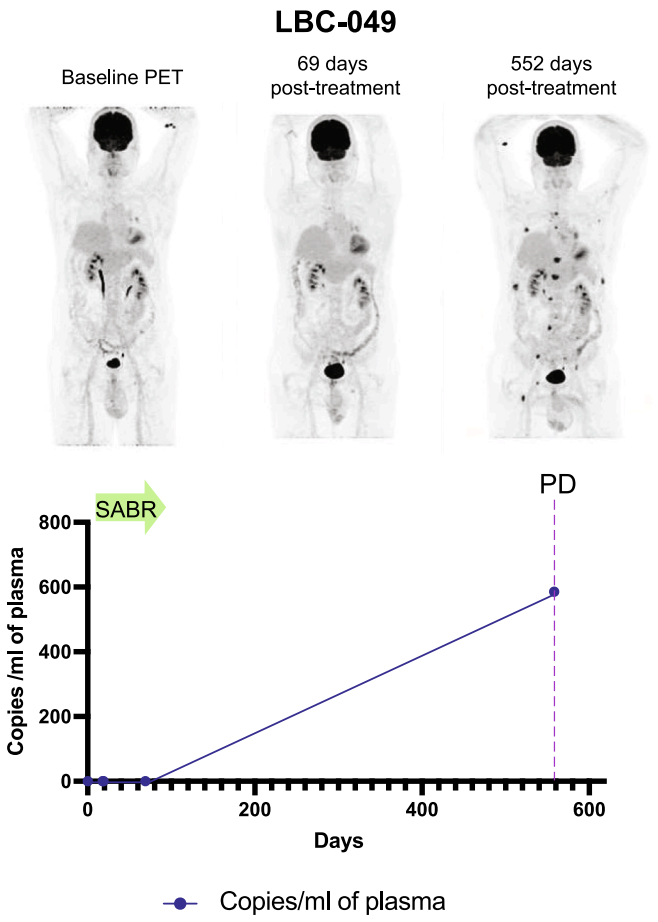

Fig. 2. ctDNA levels in a patient treated with stereotactic body ablative radiotherapy. ctDNA levels remained undetectable until progression disease was observed. SABR, stereotactic body ablative radiotherapy; PD, progressive disease.

kinetics after fractionated radiotherapy, including 25 % of cases with a notable "spike" in ctDNA levels after fraction 1.

Discussion

ctDNA is emerging as a promising non-invasive cancer biomarker. Continuing research is ongoing to help us understand its clinical validity and its potential roles in prognostication and disease monitoring. While longitudinal analysis of ctDNA as a means to track disease burden in the context of systematic treatment has been well studied, there is a paucity of data reporting early ctDNA dynamics, i.e., within hours after starting treatment. Early ctDNA dynamics may be important as increasing levels of ctDNA may correlate with tumor cell destruction and provide a surrogate biomarker of treatment response. Early ctDNA dynamics documented previously in the context of chemotherapy have varied and reports of a spike following treatment have been conflicting [5–8].

There is little information in the literature on ctDNA dynamics in curative-intent radiotherapy for NSCLC and study timepoints have varied. Walls et al. reported on 5 patients with samples at baseline and at 3 and 5 days of RT and did not observe a spike [9]. Nygard et al. reported stable ctDNA concentrations at 2 h post RT in 10 chemoRT patients [10]. Breadner et al. reported a varied NSCLC cohort, including 10 cases treated with chemoRT, with ctDNA analyses at 3, 6, 24 and 48 h post RT. They showed increases in ctDNA at 24 and 48 h [11]. In several cases, as in our own series, ctDNA became detectable only after RT. Also consistent with other studies, we observed that sequential ctDNA mirrored the disease course of our patients and gives supplementary information to that gained from imaging. Other groups have reported that baseline ctDNA levels are associated with survival [12,13].

Conclusions

In conclusion, we observed rapid changes in the early ctDNA dynamics between patients, with a spike in ctDNA levels in 3/12 (25 %) patients after the first fraction of radiotherapy, including in 2 patients with negative baseline results. While the number of patients analysed in this study is too small to conclude that a 24-hour timepoint could be used as a surrogate of tumor response, in association with the results of Breadner at al, we can conclude that rapid treatment-related changes in ctDNA occur in the first days after commencing curative intent RT in NSCLC. The mechanism(s) driving these changes are unknown. RT is known to increase tumor immunogenicity and anti-tumor responses [14] and early ctDNA changes could relate to immune clearance of dying cancer cells.

ctDNA may be represent an alternative source for mutational profiling to tumor tissue [15], and our findings have implications for selecting the optimal timepoint for testing for patients treated with radiotherapy. Testing in the first 24–48 h post RT may provide the highest yield for this still expensive diagnostic test. Our findings strongly emphasize the need to further study early ctDNA dynamics, to help

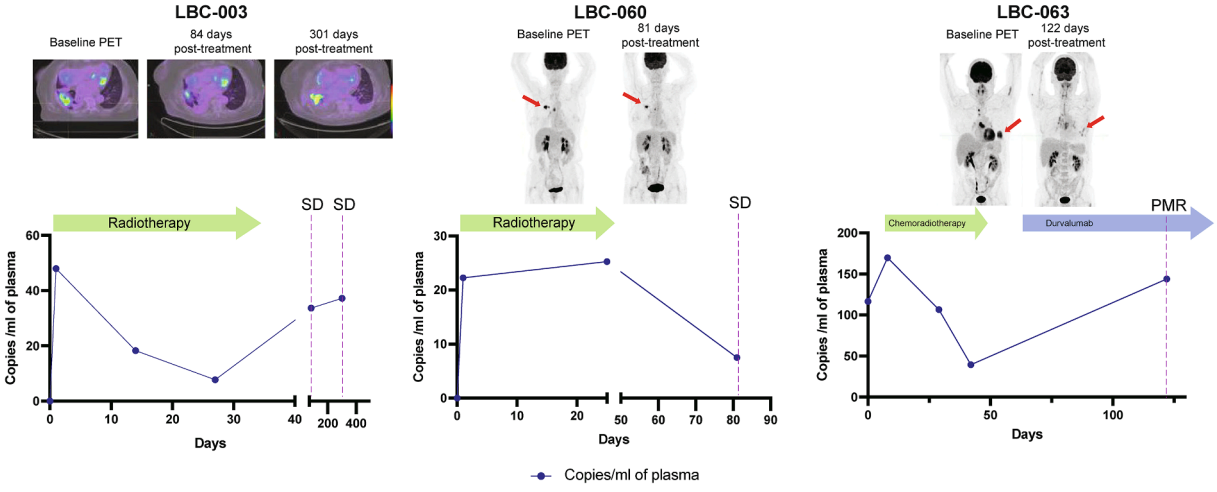

Fig. 3. Patients showing a measurable spike in ctDNA levels following radiotherapy treatment. ctDNA levels are shown for each patient with response to treatment as assessed by FDG-PET imaging indicated by the dashed line. Red arrows indicate sites of disease.

develop personalized treatment monitoring strategies for NSCLC patients.

CRediT authorship contribution statement

Michael MacManus: Methodology, Conceptualization, Formal analysis, Investigation, Resources, Funding acquisition, Project administration, Writing – original draft. **Laura Kirby:** Methodology, Investigation, Formal analysis. **Benjamin Blyth:** Conceptualization, Methodology, Investigation, Formal analysis, Project administration. **Owen Banks:** Methodology. **Olga A. Martin:** Conceptualization, Methodology, Funding acquisition. **Miriam M. Yeung:** Visualization. **Nikki Plumridge:** Methodology, Resources. **Mark Shaw:** Methodology, Resources. **Fiona Hegi-Johnson:** Methodology, Resources, Conceptualization. **Shankar Siva:** Methodology, Resources. **David Ball:** Methodology, Resources, Conceptualization, Funding acquisition. **Stephen Q. Wong:** Methodology, Conceptualization, Formal analysis, Investigation, Resources, Project administration, Writing – original draft.

Declaration of Competing Interest

FH-J has clinical trial funding, has received honoraria and participated in advisory boards for Astra Zeneca. She has received payments and honoraria from BeiGene and MSD for lectures and presentations. She receives clinical trial support from Telix Pharmaceuticals. The authors otherwise have no disclosures to report.

Acknowledgements

This work was funded through a National Health and Medical Research Council (NHMRC) project grant (ID1104139). S.Q.W is supported by an NHMRC investigator grant (#1194783). F-H. J. is supported by the Peter Mac Foundation and the Victorian Cancer Agency. Clinical trial coordinator support was provided by a grant from the Cancer council of Victoria.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctro.2023.100682.

References

[1] Mason J, Blyth B, MacManus MP, Martin OA. Treatment for non-small-cell lung cancer and circulating tumor cells. Lung Cancer Manag 2017;6(4):129–39.
[2] Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. New Engl J Med 2017;377(20):1919–29.
[3] Krebs MG, Malapelle U, André F, Paz-Ares L, Schuler M, Thomas DM, et al. Practical considerations for the use of circulating tumor DNA in the treatment of patients with cancer: a narrative review. JAMA Oncol 2022;8(12):1830.
[4] Pairawan S, Hess KR, Janku F, Sanchez NS, Mills Shaw KR, Eng C, et al. Cell-free circulating tumor DNA variant allele frequency associates with survival in metastatic cancer. Clin Cancer Res. 2020;26:1924–31.
[5] Moser T, Waldispuehl-Geigl J, Belic J, Weber S, Zhou Q, Hasenleithner SO, et al. On-treatment measurements of circulating tumor DNA during FOLFOX therapy in patients with colorectal cancer. npj Precis Oncol 2020;4(1).
[6] Riediger AL, Dietz S, Schirmer U, Meister M, Heinzmann-Groth I, Schneider M, et al. Mutation analysis of circulating plasma DNA to determine response to EGFR tyrosine kinase inhibitor therapy of lung adenocarcinoma patients. Sci Rep 2016;6(1).
[7] Tie J, Kinde I, Wang Y, Wong HL, Roebert J, Christie M, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. Ann Oncol 2015;26(8):1715–22.
[8] Patsch K, Matasci N, Soundararajan A, Diaz P, Agus DB, Ruderman D, et al. Monitoring dynamic cytotoxic chemotherapy response in castration-resistant prostate cancer using plasma cell-free DNA (cfDNA). BMC Res Notes 2019;12(1).
[9] Walls GM, McConnell L, McAleese J, Murray P, Lynch TB, Savage K, et al. Early circulating tumour DNA kinetics measured by ultra-deep next-generation sequencing during radical radiotherapy for non-small cell lung cancer: a feasibility study. Radiat Oncol 2020;15(1).
[10] Nygård L, Ahlborn LB, Persson GF, Chandrananda D, Langer JW, Fischer BM, et al. Circulating cell free DNA during definitive chemo-radiotherapy in non-small cell lung cancer patients - initial observations. PLoS One 2020;15(4):e0231884.
[11] Breadner DA, Vincent MD, Correa R, Black M, Warner A, Sanatani M, et al. Exploitation of treatment induced tumor lysis to enhance the sensitivity of ctDNA analysis: A first-in-human pilot study. Lung Cancer 2022;165:145–51.
[12] Weber S, van der Leest P, Donker HC, Schlange T, Timens W, Tamminga M, et al. Dynamic changes of circulating tumor DNA predict clinical outcome in patients with advanced non-small-cell lung cancer treated with immune checkpoint inhibitors. JCO Precis Oncol 2021;(5):1540–53.
[13] Jee J, Lebow ES, Yeh R, Das JP, Namakydoust A, Paik PK, et al. Overall survival with circulating tumor DNA-guided therapy in advanced non-small-cell lung cancer. Nat Med 2022;28(11):2353–63.
[14] Formenti SC, Rudqvist N-P, Golden E, Cooper B, Wennerberg E, Lhuillier C, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med 2018;24(12):1845–51.
[15] Heitzer E, van den Broek D, Denis MG, Hofman P, Hubank M, Mouliere F, et al. Recommendations for a practical implementation of circulating tumor DNA mutation testing in metastatic non-small-cell lung cancer. ESMO Open 2022;7(2):100399.