JLB 8 (2025) 100295

Contents lists available at ScienceDirect

The Journal of Liquid Biopsy

journal homepage: www.sciencedirect.com/journal/the-journal-of-liquid-biopsy

Circulating tumor DNA monitoring in advanced mutated melanoma (LIQUID-MEL)

Martines Gianmarco [a,‡], Palazzi Carolina [a,‡], Monica Gregorio [b], Verzè Michela [a,b], Pluchino Monica [a,b], Giudice Giulia Claire [c], Maffezzoli Michele [b,d,*], Mazzaschi Giulia [a,b], Manuguerra Roberta [e], Azzoni Cinzia [e], Bottarelli Lorena [e], Tiseo Marcello [a,b], Perrone Fabiana [a,1], Minari Roberta [a,1]

[a] Medical Oncology Unit, University Hospital of Parma, Parma, Italy
[b] Department of Medicine and Surgery, University of Parma, Parma, Italy
[c] Medical Oncology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy
[d] Portsmouth Hospital University NHS Trust, Portsmouth, United Kingdom
[e] Pathology Unit, Department of Medicine and Surgery, University of Parma, Parma, Italy

ARTICLE INFO

Keywords:
Melanoma
ctDNA
Immune checkpoint inhibitors
Prognosis
Liquid biopsy
Monitoring

ABSTRACT

Introduction: Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of metastatic melanoma, but a percentage of patients did not show benefit. Circulating tumor DNA (ctDNA) has emerged as a potential non-invasive tool for monitoring disease evolution and treatment response. The present study aimed to evaluate the clinical utility of ctDNA dynamics in patients with metastatic melanoma receiving ICIs, while exploring its role in the oncological course.

Materials and methods: The LIQUID-MEL study is a prospective, single-centre pilot study including patients with *BRAF/NRAS*-mutant metastatic melanoma. ctDNA was quantified using digital droplet PCR (ddPCR) at four different time points. Uni- and multivariable Cox regression models were used to assess the correlation between shedding and progression-free survival (PFS), and overall survival (OS).

Results: Overall, 23 patients were included. At baseline, ctDNA was detectable in 5/23 (21.7 %) cases. Baseline ctDNA shedding was associated with shorter PFS (3.88 months *vs*. 0.69 months, p=0.012). A strong numerical trend was observed also in OS (12.66 months *vs*. 2.53 months, p=0.287). Shedding at baseline did not demonstrate independent prognostic or predictive value in the uni- and multivariable analysis. The longitudinal analysis revealed intriguing patterns of ctDNA shedding in individual patients.

Conclusion: ctDNA detectability and its dynamic changes during treatment may have potential clinical utility in patients with metastatic melanoma, offering a valuable non-invasive tool for monitoring disease and treatment response. The small sample size limited the statistical power of the analysis. Further studies with larger cohorts are needed to validate its role in routine clinical practice.

1. Introduction

Long-term outcomes of patients with metastatic melanoma have substantially improved over the past decade with the advent of immune checkpoint inhibitors (ICIs), and BRAF/MEK inhibitors [1–7]. However, a considerable number of patients do not experience durable disease control, and predictive biomarkers that can be assessed prior to treatment initiation are currently lacking.

Liquid biopsy represents a promising non-invasive tool that may be used for monitoring treatment response, treatment-related adverse events and the onset of treatment resistance [8]. In this scenario, the circulating tumor DNA (ctDNA), representing fragments of DNA shed by

* Corresponding author. Department of Medicine and Surgery, University of Parma, Parma, Italy. Portsmouth Hospital University NHS Trust, Portsmouth, United Kingdom.
E-mail addresses: michele.maffezzoli@unipr.it, michele.maffezzoli@porthosp.nhs.uk (M. Michele).
‡ These authors contributed equally to this work.
[1] These authors are co-last authors.

https://doi.org/10.1016/j.jlb.2025.100295
Received 27 February 2025; Received in revised form 7 April 2025; Accepted 8 April 2025
Available online 8 April 2025
2950-1954/© 2025 The Authors. Published by Elsevier B.V. on behalf of The International Society of Liquid Biopsy. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

tumor cells into the bloodstream, offers a non-invasive means for dynamic evaluation of patients with advanced melanoma under treatment [9]. The detection and quantification of ctDNA have been promoted by the development of highly sensitive techniques, such as digital droplet PCR (ddPCR). This technology enhances the accuracy of ctDNA measurement by partitioning samples into thousands of droplets, each serving as an individual PCR reaction, thereby increasing the assay's resolution [10]. Recently, several studies investigated the role of ctDNA as a surrogate marker of tumor burden and treatment response in advanced melanoma [8,9,11].

An analysis of baseline ctDNA demonstrated that patients with detectable levels of *BRAF* p.V600E had worse overall survival (OS) and progression free survival (PFS) in response to dabrafenib or trametinib treatment compared with patients with undetectable *BRAF* p.V600E ctDNA [12]. In a separate analysis, lower levels of *BRAF* or *NRAS* ctDNA at baseline were significantly associated with response to therapy regardless of treatment received [13]. In a case series of patients with *BRAF* p.V600–mutant metastatic melanoma, ctDNA levels showed a rapid decline following initiation of dabrafenib and trametinib, becoming undetectable in 60 % of patients within six weeks and correlating with significantly better PFS [14]. Conversely, increases in *BRAF* p.V600–mutant ctDNA levels during treatment were associated with clinical progression and preceded progressive disease in 44 % of cases [15]. These findings were also confirmed in a subsequent prospective phase 2 trial [16].

Emergent data suggest that the value of ctDNA analysis extends beyond patients with *BRAF* mutations treated with target therapy. In a recent longitudinal study, ctDNA profiles were shown to be sensitive and reliable markers for treatment outcomes and early detection of primary resistance in melanoma patients harboring *BRAF* or *NRAS* mutation treated with ICIs [17–19]. Similarly, ctDNA analysis at baseline and within 12-weeks of treatment accurately differentiated pseudo-progression, defined as radiologic progression not confirmed as progressive disease upon follow-up assessment, from true disease progression [20]. ctDNA-levels were also predictive of response to ICIs: patients with undetectable ctDNA had significantly longer PFS then those with detectable ctDNA [21,22].

However, release, circulation, and detection of ctDNA may be influenced by several factors, adding layers of complexity to the analysis. Moreover, the sensitivity and specificity of ctDNA assays need improvement for routine clinical application.

The endpoint of our prospective study was to provide a dynamic view of ctDNA monitoring in patients with advanced *BRAF*- or *NRAS*-mutant melanoma treated with ICIs using ddPCR. Therefore, we monitored the quality and quantity of ctDNA at each timepoint to identify possible correlations between ctDNA shedding and oncological outcomes.

2. Materials and methods

2.1. Study design and participants

The LIQUID-MEL study is a single-centre, prospective, explorative, pilot study conducted on patients with advanced *BRAF* p.V600 or *NRAS*-mutant melanoma consecutively undergoing systemic therapy with ICIs (ipilimumab plus nivolumab or nivolumab) at the Medical Oncology Unit of the University Hospital of Parma (Italy), between August 2019 and November 2022.

Eligibility criteria included a histologically confirmed diagnosis of metastatic cutaneous melanoma harbouring *BRAF* or *NRAS* mutation, as detected through routine mutational analysis of biopsy specimens (either primary tumor or metastatic lesion if available). Patients must have been received systemic therapy with ICIs (ipilimumab plus nivolumab or nivolumab) at any treatment line for metastatic disease. Patients enrolled at the initiation of first-line therapy were also monitored during subsequent potential second-line targeted therapy, according to clinical practice. Written informed consent was obtained from all patients prior to any procedures. Exclusion criteria included synchronous malignancies aside from melanoma, treatments other than those described above or lack of available medical records.

The study procedures followed were in accordance with the declaration of Helsinki and the study was approved by the local ethical committee (229/2019/TESS/AOUPR, 14/06/2019).

2.2. Study procedures and methods

Blood samples were collected from each patient at four timepoints for each treatment line: before the beginning of systemic treatment (T0), before the administration of the second cycle of treatment (T1, 3–4 weeks), at the time of first radiological reassessment (T2, 2–3 months), at the time of the first radiological progression of disease (TPD).

Baseline *BRAF/NRAS* mutations were assessed by Easy *BRAF* and Easy *NRAS* kit (Diatech Pharmacogenetics®, Italy) following the manufacturer's instructions on tissue biopsy by the Pathology Unit of the University Hospital of Parma.

The analysis of *BRAF/NRAS* on liquid biopsy was performed on cell-free DNA (cfDNA). Blood was collected in 6 ml EDTA tubes and centrifuged twice for 10 min at 2000×*g* within 1 h after blood drawing. Plasma samples were stored at −80 °C until analysis.

cfDNA was extracted from 4 ml of plasma kit using MagCore® Super Automated Nucleic Acid Extractor (Diatech Labline).

The analysis of *BRAF/NRAS* on cfDNA was performed by *BRAF V600* screening, *NRAS Q61* screening and *NRAS G12/G13* screening ddPCR Mutation Assays (BioRad®, Hercules, CA, USA). All the procedures were executed following the specific manufacturer's instructions, at the Medical Oncology Unit of the University Hospital of Parma. QX200 Droplet Generator (Bio-Rad) was performed to generate droplets evaluate using the QX200 Droplet Reader (Bio-Rad) and data was analysed using QuantaSoft version 1.7.4. Results are presented as ctDNA fractional abundance (number of droplets positive for mutant-assay/total number of mutant- and wt-assay positive droplets). Each sample was run in triplicate and where one of these generated a total of ≤3 droplets positive for the mutation assay were defined as having ctDNA not detected.

Patients were classified as "shedders" if they exhibited detectable levels of ctDNA containing the driver mutation.

2.3. Statistical analysis

All data about the clinical history of patients, including demographic information and comorbidities, concomitant medications, melanoma diagnosis and staging, histological and molecular features, number and sites of metastasis, outcomes to anticancer treatment and advent events, clinical management, was collected.

Patients and disease characteristics were described using rates (percentages), median values and ranges. Clinical outcome was assessed in terms of objective response rate (ORR), PFS and OS. ORR was defined as the proportion of patients experiencing an objective response (either complete response or partial response) as best response to immunotherapy according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) [23]. PFS was defined as the time from systemic therapy initiation to the first documented tumor progression or death. OS was defined as the time from immunotherapy initiation until death from any cause. Patients not progressed/not died at the data cut-off of November 2022 were considered as censored at the time of the last follow-up The Kaplan–Meier method was used to estimate PFS and OS, and the log-rank test (Mantel-Cox) was applied to assess whether there were statistically significant differences in PFS and OS across subgroups. Univariable and multivariable Cox proportional hazards regression models were used to analyse the PFS and OS data. The results were expressed as Hazard Ratio (HR), 95 % confidence intervals (95 % CI), and p values. The stepwise multivariable model was developed considering only those variables with a p value < 0.10 at the univariable

Table 1
Baseline characteristics of the overall population and according to shedding at baseline (bold indicates statistically significant values). CNS: central nervous system; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ICI: immune checkpoint inhibitor; irAEs: immune-related adverse events; LDH: lactate dehydrogenase; SD: standard deviation.

Variables	Overall N = 23	No shedding at baseline N = 18 (78.3 %)	Shedding at baseline N = 5 (21.7 %)	*p*-value
Sex, n (%)	14 (60.9)	10 (55.6)	4 (80.0)	0.322
Male	9 (39.1)	8 (44.4)	1 (20.0)	
Female				
Age, mean (SD)	64.4 (10.4)	64.4 (11.0)	64.7 (9.3)	0.950
Lymphadenectomy (%)	11 (47.8)	9 (50.0)	2 (40.0)	0.692
No	12 (52.2)	9 (50.0)	3 (60.0)	
Yes				
Presence of metastases at diagnosis (%)	17 (73.9)	13 (72.2)	4 (80.0)	0.726
No	6 (26.1)	5 (27.8)	1 (20.0)	
Yes				
Type of mutation (%)	15 (65.2)	11 (61.1)	4 (80.0)	0.433
BRAF	8 (34.8)	7 (38.9)	1 (20.0)	
NRAS				
Number of metastatic sites, n (%)	10 (43.4)	9 (50.0)	1 (20.0)	0.231
<3	13 (56.6)	9 (50.0)	4 (80.0)	
≥3				
Bone metastases, n (%)	18 (78.3)	15 (83.3)	3 (60.0)	0.263
No	5 (21.7)	3 (16.7)	2 (40.0)	
Yes				
CNS metastases, n (%)	16 (69.6)	14 (77.8)	2 (40.0)	0.104
No	7 (30.4)	4 (22.2)	3 (60.0)	
Yes				
Liver metastases, n (%)	16 (69.6)	13 (72.2)	3 (60.0)	0.599
No	7 (30.4)	5 (27.8)	2 (40.0)	
Yes				
ECOG PS, n (%)	16 (69.6 %)	14 (77.8)	2 (40.0)	0.104
0	7 (30.4 %)	4 (22.2)	3 (60.0)	
1				
Previous adjuvant treatment, n (%)	16 (69.6)	12 (66.7)	4 (80.0)	0.567
No	7 (30.4)	6 (33.3)	1 (20.0)	
Yes				
ICI treatment line, n (%)	15 (65.2)	14 (77.8)	1 (20.0)	**0.016**
First-line	8 (34.8)	4 (22.2)	4 (80.0)	
Second-line				
Type of ICI, n (%)	12 (52.2)	9 (50.0)	3 (60.0)	0.692
Ipilimumab plus nivolumab	11 (47.8)	9 (50.0)	2 (40.0)	
Nivolumab				
Best response, n (%)	2 (8.7)	2 (11.1)	0 (0.0)	0.178
Complete response	7 (30.4)	7 (38.9)	0 (0.0)	
Partial response	1 (4.3)	1 (5.6)	0 (0.0)	
Stable disease	13 (56.5)	8 (44.4)	5 (100.0)	
Progressive disease				
IrAEs G3-G4, n (%)	19 (82.6)	15 (83.3)	4 (80.0)	0.862
No	4 (17.4)	3 (16.7)	1 (20.0)	
Yes				
Steroid during ICI (≥10 mg prednisone), n (%)	12 (52.2)	9 (50.0)	3 (60.0)	0.692
No	11 (47.8)	9 (50.0)	2 (40.0)	
Yes				
Target therapy after ICI, n (%)	17 (73.9)	13 (72.2)	4 (80.0)	0.726
No	6 (26.1)	5 (27.8)	1 (20.0)	
Yes				
LDH, mean (SD)	349.9 (433.8)	251.3 (88.1)	704.6 (895.0)	**0.035**

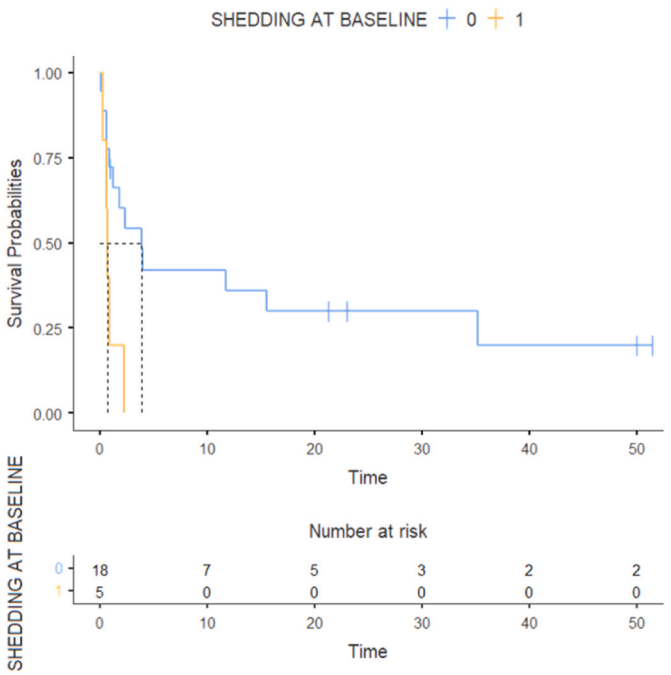

Fig. 1. Representative Kaplan-Meier survival curve illustrating the impact of shedding on progression-free survival.

analysis. The level of statistical significance was set to a value of 0.05. Logistic regression was used to assess the correlation between subgroups and the ORR.

The software JAMOVI version 2.3.21 (www.jamovi.org) was used to perform all the computational analyses and to draw the survival curves.

3. Results

3.1. Patients' characteristics

Out of the 26 patients enrolled in the study, 3 (11.5 %) were excluded due to screening failure, as no *BRAF* or *RAS* mutations were detected. Therefore, 23 patients were included in the final analysis. Baseline clinicopathological characteristics according to ctDNA shedding status, are presented in Table 1. The cohort consisted of 14 males (60.9 %), with a mean age of 64.4 years (±10.4). *BRAF* mutations were present in 15 patients (65.2 %), while 8 patients (34.8 %) harbored *NRAS* mutations. Of the *BRAF*-mutant cases, 12 patients (80 %) carried the *BRAF* p.V600E mutation. 17 patients (73.9 %) had synchronous metastatic disease at time of diagnosis. Brain involvement was observed in 7 patients (30.4 %), and 13 patients (56.6 %) had three or more metastatic sites. 16 patients (69.6 %) had not received adjuvant therapy, and 8 patients (34.8 %) received ICIs as a second-line treatment. Disease progression as best response was noted in 13 patients (56.5 %). Additionally, only half of the *BRAF* p.V600E-mutant patients received targeted therapy after ICIs, Table 1.

At baseline, ctDNA shedding was detectable in 5 patients (21.7 %). No significant differences were observed between the shedding and non-shedding groups across most variables, except for ICI treatment line and LDH. Patients in the non-shedding group were more likely to receive ICIs as first-line treatment and had lower baseline LDH levels. Supplementary Table 1 summarized blood sample collection timepoints. Among the 5 shedder patients at T0, only 1 achieved clearance at T1. The mean variant allelic frequency (VAF) at T0 was 10 % (SD 13.5). At T1, no shedding was detected, while at T2 only 2 patients were shedders, with VAFs of 2.8 % and 1.9 %. At TPD, 8 patients were shedders, with a mean VAF of 18 % (SD 15.8).

Following disease progression, only 4 patients started second-line

Table 2
Univariable and multivariable analyses for progression-free survival (bold indicates statistically significant values). CNS: central nervous system; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ICI: immune checkpoint inhibitor; irAEs: immune-related adverse events.

Variables	Patients (%)	Univariable		*p*-value	Multivariable		*p*-value
		HR	95 % CI		HR	95 % CI	
Sex, n (%)	14 (60.9)	1	[0.30–2.08]	0.635			
Male	9 (39.1)	0.79					
Female							
Lymphadenectomy (%)	11 (47.8)	1	[0.25–1.72]	0.385			
No	12 (52.2)	0.65					
Yes							
Presence of metastases at diagnosis (%)	17 (73.9)	1	[0.30–2.81]	0.881			
No	6 (26.1)	0.92					
Yes							
Type of mutation (%)	15 (65.2)	1	[0.19–1.57]	0.264			
BRAF	8 (34.8)	0.55					
NRAS							
Number of metastatic sites, n (%)	10 (43.4)	1	[0.93–6.96]	**0.070**	0.56	[0.12–2.57]	0.459
<3	13 (56.6)	2.54					
≥3							
Bone metastases, n (%)	18 (78.3)	1	[0.35–3.30]	0.908			
No	5 (21.7)	1.07					
Yes							
CNS metastases, n (%)	16 (69.6)	1	[2.18–35.52]	**0.002**	19.60	[1.74–221.03]	**0.016**
No	7 (30.4)	8.81					
Yes							
Liver metastases, n (%)	16 (69.6)	1	[0.49–4.13]	0.512			
No	7 (30.4)	1.43					
Yes							
ECOG PS, n (%)	16 (69.6 %)	1	[1.19–16.17]	**0.026**	1	[0.06–4.37]	0.553
0	7 (30.4 %)	4.39			0.53		
1							
Previous adjuvant treatment, n (%)	16 (69.6)	1	[0.33–2.37]	0.811			
No	7 (30.4)	0.89					
Yes							
ICI treatment line, n (%)	15 (65.2)	1	[1.81–14.54]	**0.002**	11.64	[1.73–78.48]	**0.012**
First-line	8 (34.8)	5.13					
Second-line							
Type of ICI, n (%)	12 (52.2)	1	[0.14–1.00]	**0.050**	0.24	[0.06–0.86]	**0.028**
Ipilimumab plus nivolumab	11 (47.8)	0.37					
Nivolumab							
IrAEs G3-G4, n (%)	19 (82.6)	1	[0.18–2.19]	0.458			
No	4 (17.4)	0.62					
Yes							
Steroid during ICI (≥10 mg prednisone), n (%)	12 (52.2)	1	[0.60–3.87]	0.381			
No	11 (47.8)	1.52					
Yes							
Target therapy after ICI, n (%)	17 (73.9)	1	[0.37–2.76]	0.975			
No	6 (26.1)	1.02					
Yes							
Shedding at baseline, n (%)	18 (78.3)	1	[1.26–13.17]	**0.019**	0.66	[0.13–3.38]	0.614
No	5 (21.7)	4.07					
Yes							

treatment with target therapy. For these patients, shedding at TPD was considered as T0 for the second-line (II) treatment (mean VAF = 13.2 %, SD 15.1). Interestingly, 2 patients had clearance at T1 (II), while another 2 patients achieved clearance at T2 (II). Finally, 2 patients had shedding at TPD (II), with a VAF of 3.2 % and 1.3 %, Supplementary Table 1.

3.2. Survival analysis in overall population and according to shedding status

The median follow-up time was 24.7 months. In the overall population, median PFS (mPFS) was 2.24 months (95 % CI: 0.89–35.2), Supplementary Fig. 1. Kaplan-Meier analysis showed a statistically significant longer mPFS in non-shedder patients compared to shedder patients at baseline (3.88 months [95 % CI: 1.18–not calculated] *vs.* 0.69 months [95 % CI: 0.66–not calculated], p=0.012), Fig. 1.

At the univariable analysis for PFS, the following factors were significantly associated with an increased risk of progression or death: three or more metastatic sites, CNS metastases, an ECOG PS of 1 compared to ECOG PS 0, receiving ICI as a second-line treatment, and baseline ctDNA shedding. At the multivariable analysis, three factors remained statistically significant: CNS involvement (HR 19.60 [95 % CI: 1.74–221.03], p=0.016), second-line ICI treatment (HR 11.64 [95 % CI: 1.73–78.48], p=0.012), and nivolumab monotherapy (HR 0.24 [95 % CI: 0.06–0.86], p=0.028). Notably, baseline ctDNA shedding did not maintain its prognostic impact in the multivariable analysis, Table 2.

In the overall population, median OS (mOS) was 4.84 months (95 % CI: 2.24–not calculated), Supplementary Fig. 2. Kaplan-Meier analysis did not show a statistically significant difference in mOS between non-shedder and shedder patients at baseline, although a strong numerical trend favoring non-shedder patients was observed (12.66 months [95 % CI: 1.51–not calculated] *vs.* 2.53 months [95 % CI: 2.27–not calculated], p=0.287), Fig. 2.

In the univariable analysis for OS, the following factors were associated with a significant increase in the risk of death: three or more metastatic sites, CNS involvement, ECOG PS of 1 compared to ECOG PS 0, and second-line ICI treatment. However, in the multivariable analysis, only ECOG PS retained significance (HR 5.65 [95 % CI: 1.20–26.54], p=0.028), Table 3.

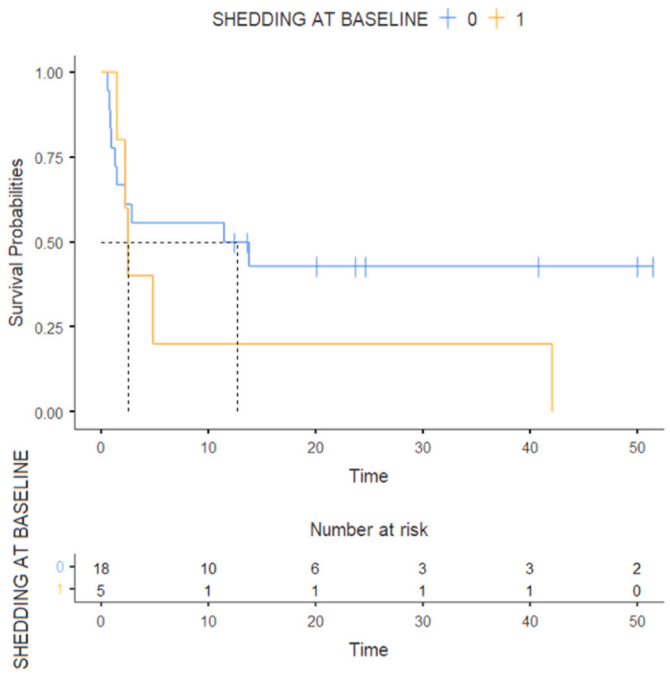

Fig. 2. Representative Kaplan-Meier survival curve illustrating the impact of shedding on overall survival.

Regarding the ORR, logistic regression analysis did not identify any variables significantly associated with treatment response.

3.3. *Longitudinal single patients' analysis*

Table 4 and Fig. 3, report a series of patients with *BRAF*p.V600 mutations and different status of ctDNA shedding at baseline. Particularly, P-08 and P-13 were non-shedder at the beginning of first-line ICI therapy. ctDNA became detectable at T2 (first imaging evaluation) and raised at the time of radiological progression. At the first-time point after the initiation of second-line target therapy (T1 II), ctDNA reverted to an undetectable state. Interestingly, P-08 had a new shedding flare at the time of radiological progression to the target therapy (TPD II). P-07 was the only shedder patient to achieve complete clearance after ICI therapy at T1. P-07 and P-23 had a flare of ctDNA shedding at T2, the first radiological evaluation, which matched with radiological disease progression. Both patients started second-line target-therapy and the subsequent timepoint (T1 II) showed an important decrease of shedding, together with a significant clinical response. Finally, at T2 (II), these patients achieved complete clearance. Interestingly, P-23 experienced another ctDNA flare at the time of radiological progression to second-line treatment (TPD II). P-10, P-12 and P-19, who were shedders at baseline, experienced a significant increase in the ctDNA between the start of ICI and the subsequent sample collected (T1), which also coincided with clinical progression of disease.

4. Discussion

Ever since the spread of ICIs, the prognosis of patients with both early-stage and advanced melanoma has significantly improved [24,25]. Unfortunately, almost a quarter of patients do not show any benefit from first-line therapy with ICIs [26], therefore making imperative the identification of prognostic and predictive biomarkers to monitor clinical course and early identify disease progression. Liquid biopsy through ctDNA monitoring is a promising and not invasive tool which could identify driver mutations, capture cancer dynamism by repeating during the course of treatment, and early detect relapse or progression [27,28]. Indeed, ctDNA levels have been related to tumor burden and response to therapy, in various malignancies, including melanoma [29–31]. Known pathogenetic genes suitable for ctDNA analysis include *BRAF*, *GNAQ*, *JAK2*, *KRAS*, *NRAS*, *MAP2K1*, *NF1, c-KIT* and *STAT1* [32]. Epigenetic changes and hypermethylation of promoter regions (such as *RARB2*, *TFPI2*, *MGMT*) are also established biomarkers, the latter helpful for an early diagnosis of melanoma [33–35]. A higher concentration of ctDNA at the time of melanoma diagnosis was associated to high tumor load and worse OS, while an increase in ctDNA shedding between baseline and following evaluations, negatively influenced survival [30,36,37].

In our prospective, explorative, pilot study, we aimed to dynamically monitor *BRAF*- or *NRAS*-mutant ctDNA of patients with advanced melanoma during systemic therapy with ICIs. Although shedding at baseline was only detectable in 5 patients (21.7 %), our results suggest that ctDNA shedding was associated with shorter PFS with a statistically significant difference between shedder and non-shedder patients (mPFS 3.88 months *vs*. 0.69 months). However, ctDNA shedding resulted not statistically significant in the multivariable analysis. No significant difference was observed in the OS analysis between the shedding and non-shedding groups, though a numerical trend favoring non-shedders was noted.

Our percentage of patients with detectable ctDNA at baseline is in line with the detection rate reported in literature, which ranges between 11 % up to 80 % [38]. This variability depends on the detection technology used, tumor burden, and the sites of metastatic disease, including brain involvement, which can ultimately reduce the fraction of ctDNA detectable in the bloodstream [29].

Our findings on the correlation between shedding and oncological outcomes are consistent with previous reports. A recent meta-analysis by Feng et al. including 617 patients with melanoma, confirmed the correlation between detectable ctDNA at diagnosis and poor survival (HR 2.91, 95 % CI: 2.22–3.82; $p < 0.001$) [39].

In our analysis, ctDNA shedding did not result as an independent prognostic factor in the multivariable analysis. Overall, multivariable analysis for PFS and OS reinforced the role of clinical factors, such as CNS involvement, ECOG PS and burden of disease as main prognostic factors. These results suggest that the prognostic value of ctDNA shedding may be overshadowed by established clinical variables, considering the limits of the small sample size.

Notably, our longitudinal analysis revealed intriguing patterns of ctDNA shedding in individual patients, especially those treated with second-line target therapy. Indeed, changes in ctDNA shedding were closely associated with treatment response and disease progression, highlighting the potential role of ctDNA as a real-time and dynamic marker of tumor behaviour. Our findings align with prior studies showing that changes in *BRAF* ctDNA levels during treatment with BRAF/MEK inhibitors can reflect radiologic response, with a decreasing related to response and a rising in the event of progression [15,37]. The level of mutant *BRAF* in ctDNA also correlated with the duration of response to target therapy, as well as with OS and PFS [40,41]. In a retrospective analysis on patients holding BRAF/MEK inhibitors due to cumulative toxicity, patients with complete response and negative ctDNA at time of discontinuation showed no progression of disease [42]. These findings were confirmed in a recent prospective study on 17 patients with stage IV melanoma treated with BRAF/MEK inhibitors [43]. The amount of ctDNA, both at baseline and during response, correlated with the type and duration of response, validating its prognostic role [43]. Additionally, the authors reported a combined approach of NGS and ddPCR assays. NGS panel showed a significant correlation with ddPCR and allowed the parallel monitoring of target genes together with the assessment of the *BRAF* copy number variation, increasing the sensitivity of the detection [43]. While *c-KIT* mutations are not directly addressed in our study, they are detected in specific melanoma subgroups, particularly in mucosal (MM), acral (AM) and chronically sun-damaged cutaneous subtypes [44]. MM and AM have a less favorable prognosis compared with cutaneous melanoma, although *c-KIT* mutations could confer sensitivity to TKIs like imatinib. However,

Table 3
Univariable and multivariable analyses for overall survival (bold indicates statistically significant values). CNS: central nervous system; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ICI: immune checkpoint inhibitor; irAEs: immune-related adverse events.

Variables	Patients (%)	Univariable		*p*-value	Multivariable		*p*-value
		HR	95 % CI		HR	95 % CI	
Sex, n (%)	14 (60.9)	1	[0.23–2.08]	0.516			
Male	9 (39.1)	0.70					
Female							
Lymphadenectomy (%)	11 (47.8)	1	[0.14–1.24]	0.116			
No	12 (52.2)	0.41					
Yes							
Presence of metastases at diagnosis (%)	17 (73.9)	1	[0.37–3.65]	0.804			
No	6 (26.1)	1.16					
Yes							
Type of mutation (%)	15 (65.2)	1	[0.22–2.22]	0.541			
BRAF	8 (34.8)	0.70					
NRAS							
Number of metastatic sites, n (%)	10 (43.4)	1	[1.03–10.35]	**0.045**	2.22	[0.59–8.38]	0.238
<3	13 (56.6)	3.26					
≥3							
Bone metastases, n (%)	18 (78.3)	1	[0.27–3.51]	0.967			
No	5 (21.7)	0.97					
Yes							
CNS metastases, n (%)	16 (69.6)	1	[1.64–17.12]	**0.002**	1.50	[0.36–6.19]	0.577
No	7 (30.4)	5.30					
Yes							
Liver metastases, n (%)	16 (69.6)	1	[0.77–6.34]	0.138			
No	7 (30.4)	2.21					
Yes							
ECOG PS, n (%)	16 (69.6 %)	1	[1.99–24.70]	**0.002**	5.65	[1.20–26.54]	**0.028**
0	7 (30.4 %)	7.02					
1							
Previous adjuvant treatment, n (%)	16 (69.6)	1	[0.16–2.04]	0.385			
No	7 (30.4)	0.57					
Yes							
ICI treatment line, n (%)	15 (65.2)	1	[1.08–8.36]	**0.035**	2.52	[0.78–8.19]	0.124
First-line	8 (34.8)	3.01					
Second-line							
Type of ICI, n (%)	12 (52.2)	1	[0.20–1.67]	0.307			
Ipilimumab plus nivolumab	11 (47.8)	0.57					
Nivolumab							
IrAEs G3-G4, n (%)	19 (82.6)	1	[0.04–2.19]	0.228			
No	4 (17.4)	0.29					
Yes							
Steroid during ICI (≥10 mg prednisone), n (%)	12 (52.2)	1	[0.30–2.50]	0.789			
No	11 (47.8)	0.86					
Yes							
Target therapy after ICI, n (%)	17 (73.9)	1	[0.22–2.19]	0.531			
No	6 (26.1)	0.69					
Yes							
Shedding at baseline, n (%)	18 (78.3)	1	[0.60–5.33]	0.294			
No	5 (21.7)	1.79					
Yes							

Table 4
Dynamic changes of ctDNA for representative patients. T0, start of systemic treatment; T1, before second cycle (3–4 weeks); T2, first radiological reassessment (2–3 months); TPD, radiological progression of disease (TPD in a cell denotes progression timepoint); D, death before or near the collection timepoint; (II), second-line treatment.

	Mutation	T0	T1	T2	TPD	T0 (II)	T1 (II)	T2 (II)	TPD (II)
P-07	***BRAF* p.V600E**	**0.11 %**	**WT**	TPD	**5.50 %**	**5.50 %**	**0.17 %**	**WT**	**-**
P-08	***BRAF* p.V600K**	**WT**	**WT**	**2.80 %**	**5.50 %**	**5.50 %**	**WT**	TPD (II)	**3.20 %**
P-10	***BRAF* p.V600E**	**1.83 %**	TPD	TPD	**35.00 %**	D			
P-12	***BRAF* p.V600E**	**30.00 %**	TPD	TPD	**37.00 %**	D			
P-13	***BRAF* p.V600K**	**WT**	**WT**	**1.90 %**	**6.00 %**	**6.00 %**	**WT**	**-**	**-**
P-19	***BRAF* p.V600K**	**0.12 %**	TPD	TPD	**19.00 %**	D			
P-23	***BRAF* p.V600E**	**WT**	**WT**	TPD	**35.80 %**	**35.80 %**	**0.34 %**	**WT**	**1.30 %**

response depends on mutation type and anatomical site [45]. Liquid biopsy enables real-time monitoring of *c-KIT* mutational status, capturing clonal evolution and resistance mechanisms during therapy. Therefore, tracking ctDNA in these patients could inform therapeutic decisions, such as switching TKIs or combining with ICIs, based on emerging resistance [45]. In this scenario, ongoing studies are investigating the role of TKIs combined with ICIs in *c-KIT* mutated melanoma [44].

Regarding ctDNA role in patients receiving ICIs, multiple data confirmed its feasibility as a marker of response, exceeding LDH and

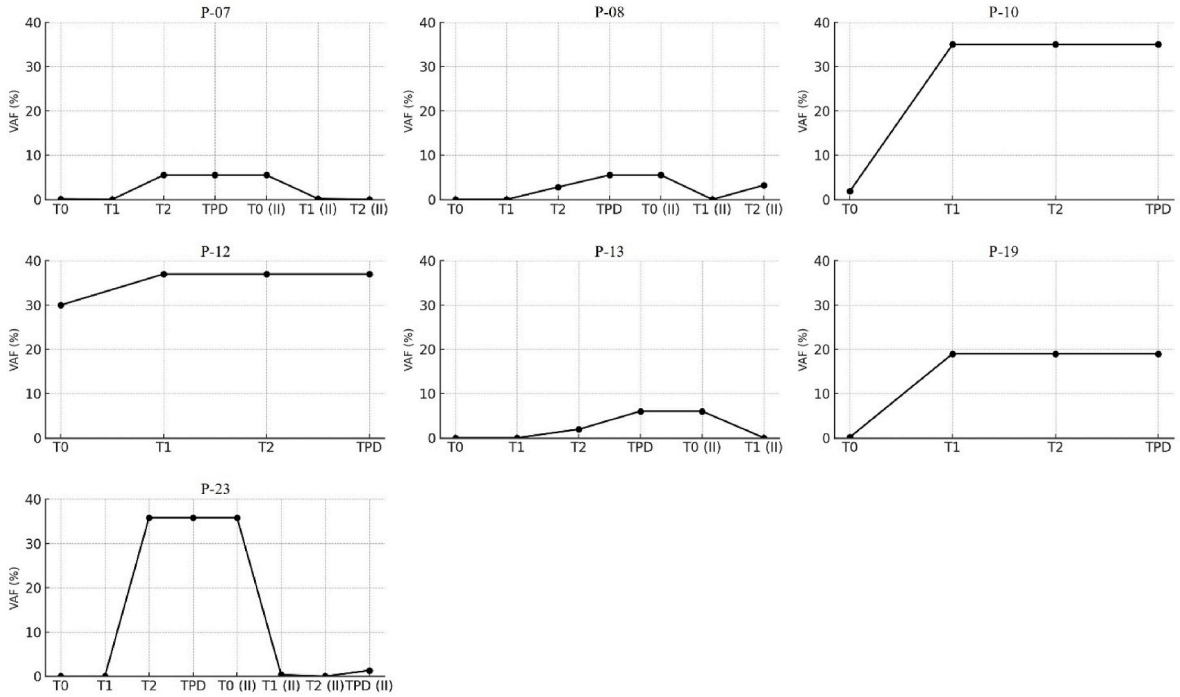

Fig. 3. Dynamic changes of ctDNA for representative patients. T0, start of systemic treatment; T1, before second cycle (3–4 weeks); T2, first radiological reassessment (2–3 months); TPD, radiological progression of disease (TPD in a cell denotes progression timepoint); D, death before or near the collection timepoint; (II), second-line treatment.

S100 dynamics [46]. Prospective evidence in patients with stage III and IV melanoma demonstrated that ctDNA levels correlated better with tumor burden and metabolic activity, measured as metabolic tumor volume (MTV) on PET/CT, than LDH or S100 [47]. Furthermore, early changes in ctDNA levels after treatment initiation accurately predicted RECIST response and were associated with improved OS and PFS, showing higher sensitivity and both negative and positive predictive values compared to LDH and S100 [48].In our longitudinal single patient's analysis, only 1 patient achieved complete clearance during ICI therapy. Intriguingly, ctDNA flares during treatment with ICIs corresponded to clinical and radiological progression. ctDNA detection rates were in line with the burden of disease, with lower detection at early timepoints (T0 = 21.7 %, T1 = 22.2 %) and higher detection at progression, when shedding is more likely (TPD = 44.4 %, TPD (II) = 50 %), Supplementary Table 1. According to our findings, several studies described the ability of ctDNA shedding to reflect the behaviour of the disease in patients with advanced melanoma receiving ICIs [18,30,49]. In these patients, a decreasing in ctDNA during treatment was associated with better response and survival [17], and an enduring decrease was also related to prolonged radiological responses [50]. Conversely, an increase in ctDNA during ICIs was able to predict disease progression about 3.5 months earlier than radiologic imaging [51]. These findings were confirmed in a prospective trial on 48 patients, where ctDNA reduction during ICIs were associated with favorable survival outcomes [9]. Interestingly, for each unit rise in ctDNA shedding from baseline, an increased risk of PD of 24 % was described [9]. Further confirmatory results come from another recent prospective study that enrolled 104 patients receiving palliative and adjuvant ICIs [47]. A decrease of variant allele fractions correlated with metabolic response on PET and improved survival outcomes in the palliative cohort. Additionally, ctDNA monitoring detected early recurrence in the adjuvant cohort, therefore paving the way for its use in this setting [47]. Similarly to what described with BRAF/MEK inhibitors, a lower DNA level at time of ICIs holding after durable response, was related to longer treatment-free interval [52]. Finally, longitudinal ctDNA monitoring was able to distinguish between pseudo-progression and radiological true PD to ICIs, showing a sensitivity and specificity for predicting pseudo-progression of 90 % (95 %CI, 68 %–99 %) and 100 % (95 %CI, 60 %–100 %), respectively [20,53].

Our study has major limitations. First, the small sample size and the limited percentage of patients with ctDNA shedding affected the statistical power of the analysis, as well as reliability, causality and generalizability of results. This represents the main limitation of our study, together with the single-centre design, which limits the external validity of our results. Additionally, the study was conducted using ddPCR and not NGS, therefore missing information on tumor heterogeneity and potential mechanisms of resistance. Indeed, tumor heterogeneity, differences in metastatic sites, use of different ICIs, and variations in ctDNA extraction protocols could influence ctDNA levels and their interpretation.

Several pre-analytical variables may have influenced ctDNA detection. Temporal variability of liquid biopsy collection, delayed plasma separation and centrifugation, different centrifugation speed and low plasma volumes, as well as limitations inherent to the ddPCR assay, particularly its targeted nature and threshold for positivity, may have impaired sensitivity and detection rates. These observations highlight the need for standardized pre-analytical protocols, including collection tubes, immediate processing, adequate plasma volumes, and centrifugation steps. At the analytical level, consensus parameters on assay sensitivity, detection limits and sample quality should be validated to support broader implementation of liquid biopsy. Eventually, as sequencing costs decrease, tumor-uninformed NGS-based approaches hold higher sensitivity and broader mutation panels. Integration of ctDNA into clinical practice requires not only analytical validation, but also robust demonstration of clinical utility. Randomized prospective trials are essential to determine whether clinical decision making based on ctDNA could improve treatment outcomes compared to standard monitoring and guide personalized treatment strategies [54].

5. Conclusion

Despite the above limitations, our study reinforced the potential

utility of ctDNA as a dynamic biomarker for disease monitoring in real-world patients with advanced melanoma, and the use of ddPCR for highly sensitive analysis. These data need to be further validated in larger, multicentre prospective trials.

Consent to participate

The obtainment of informed consent for live patients was mandatory. Informed consent was obtained from all individual participants included in the study.

Compliance with ethical standards/ethics approval

The study was conducted following the approval by the Local ethics committee (229/2019/TESS/AOUPR, 14/06/2019)). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Author contributions

All Authors contributed to the conception and design of the study, collecting and curating data, drafting, editing and revising the manuscript critically. Michele Maffezzoli has also given substantial contribution to data analysis and methodology. Michele Maffezzoli, Roberta Minari, Fabiana Perrone and Giulia Mazzaschi has also contributed to supervision, validation and visualization of the manuscript. All authors read and approved the final version of the manuscript. All authors have sufficiently participated to the study and agreed to be accountable for all aspects of the work.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Declaration of conflict interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None. The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2025.100295.

References

[1] Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363(8):711–23.
[2] Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without *BRAF* mutation. N Engl J Med 2015;372(4):320–30.
[3] Schachter J, Ribas A, Long GV, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet 2017;390(10105):1853–62.
[4] Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. J Clin Oncol 2022;40(2):127–37.
[5] Long GV, Flaherty KT, Stroyakovskiy D, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. Ann Oncol 2017;28(7):1631–9.
[6] Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAFV600-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol 2016;17(9):1248–60.
[7] Dummer R, Flaherty KT, Robert C, et al. COLUMBUS 5-year update: a randomized, open-label, phase III trial of encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with *BRAF* V600–mutant melanoma. J Clin Oncol 2022;40(36):4178–88.
[8] Calapre L, Warburton L, Millward M, et al. Circulating tumour DNA (ctDNA) as a liquid biopsy for melanoma. Cancer Lett 2017;404:62–9.
[9] Di Nardo L, Del Regno L, Di Stefani A, et al. The dynamics of circulating tumour DNA (ctDNA) during treatment reflects tumour response in advanced melanoma patients. Exp Dermatol 2023;32(10):1785–93.
[10] Chang-Hao Tsao S, Weiss J, Hudson C, et al. Monitoring response to therapy in melanoma by quantifying circulating tumour DNA with droplet digital PCR for BRAF and NRAS mutations. Sci Rep 2015;5(1):11198.
[11] Marsavela G, Johansson PA, Pereira MR, et al. The prognostic impact of circulating tumour DNA in melanoma patients treated with systemic therapies—beyond BRAF mutant detection. Cancers (Basel) 2020;12(12):3793.
[12] Santiago-Walker A, Gagnon R, Mazumdar J, et al. Correlation of *BRAF* mutation status in circulating-free DNA and tumor and association with clinical outcome across four BRAFi and MEKi clinical trials. Clin Cancer Res 2016;22(3):567–74.
[13] Gray ES, Rizos H, Reid AL, et al. Circulating tumor DNA to monitor treatment response and detect acquired resistance in patients with metastatic melanoma. Oncotarget 2015;6(39):42008–18.
[14] Schreuer M, Meersseman G, van Den Herrewegen S, et al. Applications for quantitative measurement of BRAF V600 mutant cell-free tumor DNA in the plasma of patients with metastatic melanoma. Melanoma Res 2016;26(2):157–63.
[15] Schreuer M, Meersseman G, Van Den Herrewegen S, et al. Quantitative assessment of BRAF V600 mutant circulating cell-free tumor DNA as a tool for therapeutic monitoring in metastatic melanoma patients treated with BRAF/MEK inhibitors. J Transl Med 2016;14(1):95.
[16] Schreuer M, Jansen Y, Planken S, et al. Combination of dabrafenib plus trametinib for BRAF and MEK inhibitor pretreated patients with advanced BRAFV600-mutant melanoma: an open-label, single arm, dual-centre, phase 2 clinical trial. Lancet Oncol 2017;18(4):464–72.
[17] Lee JH, Long GV, Boyd S, et al. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. Ann Oncol 2017;28(5):1130–6.
[18] Ashida A, Sakaizawa K, Uhara H, et al. Circulating tumour DNA for monitoring treatment response to anti-PD-1 immunotherapy in melanoma patients. Acta Derm Venereol 2017;97(10):1212–8.
[19] Lee JH, Long GV, Menzies AM, et al. Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti–programmed cell death 1 antibodies. JAMA Oncol 2018;4(5):717.
[20] Lee JH, Long GV, Menzies AM, et al. Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti–programmed cell death 1 antibodies. JAMA Oncol 2018;4(5):717.
[21] Pedersen JG, Madsen AT, Gammelgaard KR, et al. Inflammatory cytokines and ctDNA are biomarkers for progression in advanced-stage melanoma patients receiving checkpoint inhibitors. Cancers (Basel) 2020;12(6):1414.
[22] Seremet T, Jansen Y, Planken S, et al. Undetectable circulating tumor DNA (ctDNA) levels correlate with favorable outcome in metastatic melanoma patients treated with anti-PD1 therapy. J Transl Med 2019;17(1):303.
[23] Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45(2):228–47.
[24] Sheng F, Yan Y, Zeng B. Efficacy and safety of immune checkpoint inhibitors and targeted therapies in resected melanoma: a systematic review and network meta-analysis. Front Pharmacol 2023;14.
[25] Singh SRK, Malapati SJ, Kumar R, et al. NCDB analysis of melanoma 2004–2015: epidemiology and outcomes by subtype, sociodemographic factors impacting clinical presentation, and real-world survival benefit of immunotherapy approval. Cancers (Basel) 2021;13(6):1455.
[26] Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol 2018;19(11):1480–92.
[27] Blanco BA, Wolfgang CL. Liquid biopsy for the detection and management of surgically resectable tumors. Langenbecks Arch Surg 2019;404(5):517–25.
[28] Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. J Hematol Oncol 2022;15(1):131.
[29] Pikturniene R, Cesas A, Jarmalaite S, et al. Harnessing ctDNA in advanced melanoma: a promising tool for informed clinical decisions. Cancers (Basel) 2024;16(6):1197.
[30] Forschner A, Battke F, Hadaschik D, et al. Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma – results of a prospective biomarker study. J Immunother Cancer 2019;7(1):180.
[31] Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6(224).

[32] Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012;150(2):251–63.
[33] Diefenbach RJ, Lee JH, Rizos H. Methylated circulating tumor DNA as a biomarker in cutaneous melanoma. Melanoma Manag. 2020;7(3).
[34] Eslami-S Z, Cortés-Hernández LE, Cayrefourcq L, et al. The different facets of liquid biopsy: a kaleidoscopic view. Cold Spring Harb Perspect Med. 2020;10(6): a037333.
[35] Hodgson DR, Wellings R, Orr MCM, et al. Circulating tumour-derived predictive biomarkers in oncology. Drug Discov Today 2010;15(3–4):98–101.
[36] Wong SQ, Raleigh JM, Callahan J, et al. Circulating tumor DNA analysis and functional imaging provide complementary approaches for comprehensive disease monitoring in metastatic melanoma. JCO Precis Oncol 2017;(1):1–14.
[37] Sanmamed MF, Fernández-Landázuri S, Rodríguez C, et al. Quantitative cell-free circulating BRAFV600E mutation analysis by use of droplet digital PCR in the follow-up of patients with melanoma being treated with BRAF inhibitors. Clin Chem. 2015;61(1):297–304.
[38] Zheng Y, Sun H, Cong L, et al. Prognostic value of ctDNA mutation in melanoma: a meta-analysis. JAMA Oncol 2021;2021:1–13.
[39] Feng SN, Cen XT, Tan R, et al. The prognostic value of circulating tumor DNA in patients with melanoma: a systematic review and meta-analysis. Transl Oncol 2021;14(6):101072.
[40] Syeda MM, Wiggins JM, Corless BC, et al. Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study. Lancet Oncol 2021;22(3):370–80.
[41] Chang GA, Tadepalli JS, Shao Y, et al. Sensitivity of plasma BRAF ^{mutant} and NRAS ^{mutant} cell-free DNA assays to detect metastatic melanoma in patients with low RECIST scores and non-RECIST disease progression. Mol Oncol 2016;10(1): 157–65.
[42] Di Guardo L, Randon G, Corti F, et al. Liquid biopsy and radiological response predict outcomes following discontinuation of targeted therapy in patients with *BRAF* mutated melanoma. Oncologist 2021;26(12):1079–84.
[43] Scaini MC, Catoni C, Poggiana C, et al. A multiparameter liquid biopsy approach allows to track melanoma dynamics and identify early treatment resistance. npj Precis Oncol 2024;8(1).
[44] Steeb T, Wessely A, Petzold A, et al. c-Kit inhibitors for unresectable or metastatic mucosal, acral or chronically sun-damaged melanoma: a systematic review and one-arm meta-analysis. Eur J Cancer 2021;157:348–57.
[45] Pham DDM, Guhan S, Tsao H. KIT and melanoma: biological insights and clinical implications. Yonsei Med J 2020;61(7):562–71.
[46] Tolmeijer SH, Koornstra RHT, de Groot JWB, et al. Plasma BRAF mutation detection for the diagnostic and monitoring trajectory of patients with LDH-high stage IV melanoma. Cancers (Basel) 2021;13(15):3913.
[47] Schroeder C, Gatidis S, Kelemen O, et al. Tumour-informed liquid biopsies to monitor advanced melanoma patients under immune checkpoint inhibition. Nat Commun 2024;15(1):8750.
[48] Braune J, Keller L, Schiller F, et al. Circulating tumor DNA allows early treatment monitoring in BRAF- and NRAS-mutant malignant melanoma. JCO Precis Oncol 2020;(4):20–31.
[49] Seremet T, Planken S, Schreuer M, et al. Illustrative cases for monitoring by quantitative analysis of BRAF/NRAS ctDNA mutations in liquid biopsies of metastatic melanoma patients who gained clinical benefits from anti-PD1 antibody therapy. Melanoma Res 2018;28(1):65–70.
[50] Herbreteau G, Vallée A, Knol A-C, et al. Circulating tumor DNA early kinetics predict response of metastatic melanoma to anti-PD1 immunotherapy: validation study. Cancers (Basel) 2021;13(8):1826.
[51] Váraljai R, Wistuba-Hamprecht K, Seremet T, et al. Application of circulating cell-free tumor DNA profiles for therapeutic monitoring and outcome prediction in genetically heterogeneous metastatic melanoma. JCO Precis Oncol 2019;(3):1–10.
[52] Warburton L, Calapre L, Pereira MR, et al. Circulating tumour DNA in advanced melanoma patients ceasing PD1 inhibition in the absence of disease progression. Cancers (Basel) 2020;12(11):3486.
[53] Takai E, Omata W, Totoki Y, et al. Clonal dynamics of circulating tumor DNA during immune checkpoint blockade therapy for melanoma. Cancer Sci 2021;112 (11):4748–57.
[54] Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic — implementation issues and future challenges. Nat Rev Clin Oncol 2021;18(5): 297–312.