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RESEARCH ARTICLE

The dynamics of circulating tumour DNA (ctDNA) during treatment reflects tumour response in advanced melanoma patients

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Abstract

Despite the introduction of targeted (BRAFi/MEKi) and immune checkpoint inhibitors (ICIs) has significantly reduced the recurrence rate and improved the overall survival (OS) of patients with Stage III and IV melanoma, only a percentage will benefit of durable disease control. The aim of this study was to examine whether the levels of circulating tumour DNA (ctDNA) in plasma of advanced melanoma patients undergoing BRAFi/MEKi or ICIs vary according to the patients' survival outcomes (i.e. progression-free survival (PFS) and OS) and disease progression. Plasma samples of Stage III-IV melanoma patients were collected at baseline (treatment initiation) and thereafter every 3 months. Circulating $BRAF^{V600E/K}$ and $NRAS^{Q61R/K}$ mutations were analysed through droplet digital PCR (ddPCR, Bio-Rad) in a total of 177 plasma samples from 48 melanoma patients (19 Stage III, 29 Stage IV). Baseline ctDNA concentration was significantly associated with OS (HR = 1.003, 95% CI = 1.000–1.006, $p = 0.043$) and PFS (HR = 1.004, 95% CI = 1.000–1.007, $p = 0.029$) independent of clinical-prognostic confounders. For each unit increase in the ΔctDNA (concentration difference between

the last follow-up and baseline) there was a 24% increased risk of disease progression, irrespective of treatment type and stage at diagnosis (OR = 1.24, 95% CI = 1.03–1.49, $p = 0.020$, AUC = 0.93). Patients with reduction of ctDNA level from baseline to the last follow-up had longer OS (HR = 0.14; 95% CI = 0.05–0.44, $p = 0.001$) and PFS (HR = 0.08; 95% CI = 0.03–0.27, $p < 0.0001$) compared to patients with increased ctDNA, including adjustment for confounding factors. Our findings suggest that variation of ctDNA over time during melanoma treatment reflects the clinical outcome and tumour response to therapy and might be helpful in clinical monitoring.

KEYWORDS
circulating tumour DNA, follow-up studies, immune checkpoint inhibitors, melanoma, progression-free survival

1 | INTRODUCTION

The incidence of cutaneous melanoma (CM) is increasing in the white population, with 9–19 new melanoma cases per 100 000 inhabitants in Europe, leading to more than 57 000 new deaths from melanoma worldwide in 2020.[1,2] Early diagnosis and surgical excision of Stage I and II CM show a 10-year survival rate of 75%–90%. The clinical prognosis of Stage III melanoma is rather heterogeneous, with a 10-year melanoma-specific survival ranging from 88% to 24% for Stage IIIA and IIID patients, respectively.[3,4] The continuous evolution of new immunotherapies or combination treatments (ipilimumab, nivolumab, pembrolizumab and combination ipilimumab/nivolumab), anti-BRAF and anti-MEK combined therapy (dabrafenib/trametinib, vemurafenib/cobimetinib, encorafenib/binimetinib) for patients with metastatic melanoma and then also in the adjuvant setting has dramatically improved the survival outcome of advanced melanoma patients from a median survival of 9 months before 2011 to about 2 years.[5–7] However, the best sequencing or combination approach remains an open question,[8] and biomarker analyses are ongoing to refine patient stratification, monitor treatment efficacy and disease progression, and define the most appropriate therapeutic strategies.

Although with promising preliminary results,[9,10] circulating RNAs were investigated as potential biomarkers of disease onset and spread, without conclusive results. Contrastingly, circulating tumour DNA (ctDNA) has been extensively explored as a prognostic and predictive biomarker, and for disease burden monitoring across several cancer types. ctDNA derives from apoptotic and necrotic cancer cells, as well as active tumour secretion, and retains the genetic and epigenetic changes of the parental cancer cell, thereby reflecting the tumour molecular profile.[11] Somatic mutations in *BRAF* and *NRAS* driver genes account for 70% of all cutaneous melanoma diagnoses (prevalence of 50% and 25%, respectively)[12]; therefore, CM represents an ideal setting for implementing mutant ctDNA analysis as a prognostic and predictive biomarker.[13] Recent studies explored the role of ctDNA in Stage III and IV melanoma patients treated with BRAFi/MEKi and immune checkpoint inhibitors (ICIs), where an association between ctDNA levels and patients' clinical outcomes was found.[14–17] Nevertheless, the majority of these studies were retrospective in design, and limited to pretreatment and/or early on treatment ctDNA analysis (range: 4–12 weeks).

The aim of our study was to prospectively investigate whether plasma ctDNA changes over time could predict survival outcomes and tumour response in a cohort of Stage III and IV melanoma patients treated with BRAFi/MEKi or ICI.

2 | MATERIALS AND METHODS

2.1 | Study design

A multicentre prospective observational study was performed at the Dermatology Clinic of Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome (Italy), and of the University of L'Aquila, Ospedale San Salvatore, L'Aquila (Italy), from December 2018 to February 2020. Patients aged ≥18 years, diagnosed with Stage III or IV melanoma, who were treatment-naïve and eligible for BRAFi/MEKi or ICI as per decision of the local Multidisciplinary Tumor Board, entered the study at the time of treatment initiation. The present study was approved by the ethics committees of the involved institutions (Ethical Committee Fondazione Policlinico Universitario A. Gemelli-IRCCS, Università Cattolica del Sacro Cuore, Prot N: 2044) and followed the ethical standards on human experimentation (institutional or regional) as well as the Helsinki Declaration. The patients in this manuscript have given written informed consent to the publication of their case details.

Baseline patients' clinical characteristics and histopathological features of melanoma were registered, including sex, age at tumour diagnosis, baseline LDH serum levels, Eastern Cooperative Oncology Group (ECOG) performance status, primary tumour *BRAF* and *NRAS* mutational status, number and location of lymph node and distant organ metastatic sites, type of first-line therapy, tumour histopathological subtype and Breslow thickness (Table 1).

The primary end points were progression-free survival (PFS) and overall survival (OS), defined as the time from treatment initiation until disease progression or death from any cause, respectively.

TABLE 1 Baseline characteristics of patients and tumours according to ctDNA baseline detectability.

	Total $N = 48$ (%)[a]	Detectable ctDNA at baseline $N = 19$ (%)[a]	Undetectable ctDNA at baseline $N = 29$ (%)[a]	p Value*
Sex				
Male	29 (60.4)	8 (42.1)	11 (37.9)	0.772
Female	19 (39.6)	11 (57.9)	18 (62.1)	
Median age (IQR)	57 (45.5–76.5)	59 (53–77)	52 (40–70)	0.145
LDH[b]				
≤ULN	34 (70.8)	12 (63.2)	22 (75.9)	0.498
>ULN	5 (10.4)	1 (5.3)	4 (13.8)	
Mean LDH (±SD)	248.3 (115.7)	253.3 (110.6)	245.6 (120.4)	0.845
ECOG				
0	39 (81.2)	15 (78.9)	24 (82.8)	0.832
1	6 (12.5)	3 (15.8)	3 (10.3)	
2	2 (4.2)	1 (5.3)	1 (3.4)	
Clinical staging				
III	19 (39.6)	5 (26.3)	14 (48.3)	0.128
IV	29 (60.4)	14 (73.7)	15 (51.7)	
Distant metastases				
M0	19 (39.6)	5 (26.3)	14 (48.3)	0.166
M1a	10 (25.0)	5 (26.3)	5 (17.2)	
M1b	5 (10.4)	2 (10.5)	3 (10.3)	
M1c	11 (22.9)	7 (36.8)	4 (13.8)	
M1d	3 (6.3)	0	3 (10.3)	
Histological subtypes				
SSM	16 (33.3)	5 (26.3)	11 (37.9)	0.405
NM	17 (35.4)	7 (36.8)	10 (34.5)	
Spitzoid	4 (8.3)	3 (15.8)	1 (3.4)	
ALM	1 (2.1)	1 (5.3)	0	
Occult	4 (8.3)	2 (10.5)	2 (6.9)	
na	6 (12.5)	1 (5.3)	5 (17.2)	
Breslow thickness				
<5 mm	29 (60.4)	14 (73.7)	15 (51.7)	0.083
≥5 mm	11 (22.9)	2 (10.5)	9 (31.0)	
na	8 (16.7)	3 (15.8)	5 (17.2)	
Ulceration				
Present	25 (52.1)	14 (73.7)	11 (37.9)	**0.003***
Absent	18 (37.5)	2 (10.5)	16 (55.2)	
na	5 (10.4)	3 (15.8)	2 (6.9)	
Number of metastatic sites				
<2	35 (72.9)	13 (68.4)	22 (75.9)	0.571
≥2	13 (27.1)	6 (31.6)	7 (24.1)	

Abbreviations: ALM, acral lentiginous melanoma; na, data not available; NM, nodular melanoma; SSN, superficial spreading melanoma.

[a]Numbers do not always add up to the total due to missing data.

[b]LDH > ULN, serum lactate dehydrogenase level greater than upper limited of normal.

*Statistically significant p values are in bold.

Secondary end points were investigator-assessed objective response rate (ORR), defined as the proportion of patients who had a confirmed tumour response measured according to Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1. Extra- and intracranial tumour response assessment was performed at a time interval of 4–6 months, depending on patients' individual risk factors and tumour burden. Patients were grouped in four categories based on their best objective tumour response: complete remission (CR), partial response (PR) defined as a decrease of at least 30% in the extent of the tumour, stable disease (SD) and progressive disease (PD). Patients lacking restaging imaging following clinical disease progression were excluded from the analysis.

2.2 | Experimental procedure

Twelve millilitres of peripheral whole blood were collected in ethylenediaminetetraacetic acid (EDTA) tubes prior to treatment initiation, and subsequently every 3 months during therapy until patient death or withdrawal from the study. Plasma samples were processed within 4 h from collection, by gently inverting and then centrifuging the EDTA tubes at 1900 rpm for 10 min, followed by additional plasma supernatant centrifugation for 10 min at 8765 *g* before recovery and storage at −80°C until use. Plasma samples were further centrifuged for 10 min at 8765 *g*, immediately prior to ctDNA extraction using the QIAamp Circulating Nucleic Acid Kit (Qiagen), according to the manufacturer's procedure. Total eluate from each sample was divided into two replicate wells, and the concentration of the $BRAF^{V600E/K}$ and $NRAS^{Q61R/K}$ was measured using droplet digital PCR (ddPCR, Bio-Rad) assays (assay numbers: dHsaMDV2010035, dHsaMDV2010027, dHsaMDV2010067, dHsaMDV2010071) on the QX200™ Droplet Digital PCR system (Bio-Rad). Droplets were read individually on the QX200™ droplet reader (Bio-Rad). The normal assay range was determined using plasma samples from 10 healthy donors and the limit of blank (LOB) was measured (LOB = mean blank [from 10 healthy volunteers] + 1.645*SD blank [of three replicates])[18] as indicative of the false positivity rate and specificity of each mutant assay, considering detectable those ctDNA concentration above the corresponding LOB. Both BRAF assays (V600E/K) showed no mutant copies in any healthy donors, so the LOB was 0 copies/mL. NRAS assays had LOB of 0.4 copies/mL and 0.6 copies/mL for Q61R and Q61K, respectively. Data were analysed using QuantaSoft™ software version 1.6.6.0320.

The number of mutated DNA copies per reaction derived from QuantaSoft analysis was used to calculate the ctDNA copies per mL of plasma, considering the volume of plasma used for ctDNA extraction (mL), the volume (μL) in which ctDNA was eluted and the volume (μL) of ctDNA added to PCR reaction.

2.3 | Statistical analysis

Baseline patients and tumour characteristics (sex, age, AJCC stage, number of metastatic sites, LDH concentration, primary tumour Breslow thickness and ulceration) were analysed according to baseline ctDNA (detectable/undetectable) using Fisher's exact test. Response assessment was referred to the last time point of plasma collection for each patient, defining two subgroups: non-responders (PD/SD) and responders (CR/PR). Association between baseline ctDNA copy number (considered both as categorical variables: detectable/undetectable, and continuous variable), ctDNA copy number variation during therapy (considered as categorical variables: increase vs. decrease/no change from baseline to the last time point, namely T0–Tlast, of blood collection) and PFS and OS was investigated using a multivariable Cox regression model, including clinical prognostic factors as covariates (number of distant organ metastatic sites, age, baseline circulating mutational status and systemic therapy received). The Kaplan–Meier method was used to calculate the median PFS, the log-rank test was performed for comparison and a Cox model was fitted to estimate the hazard ratio (HR).

A multivariable logistic regression model was built to investigate the predictive role of ΔctDNA concentration (measured as the difference in ctDNA concentration between the last time point of plasma collection and baseline) on RECIST outcome during therapy, including stage at diagnosis and type of systemic therapy received as covariates. The ability of the model to predict progressive/stable disease or complete/partial tumour response was estimated using the receiver operator characteristic (ROC) curve analysis and calculation of the area under the curve (AUC). The quality of the model and the goodness-of-fit was defined through the likelihood ratio (LR) and Hosmer–Lemeshow tests, respectively.

All statistical analyses were conducted using STATA/BE v.17 (StataCorp LLC), considering statistically significant p values < 0.05.

3 | RESULTS

3.1 | Patients' characteristics

Forty-eight advanced cutaneous melanoma patients, including 29/48 (60.4%) men and 19/48 (39.6%) women with a median age of 57 years (IQR = 45.5–76.5), were included in the study. Disease stage was as follows: 29/48 (60.4%) patients had distant metastases (Stage IV), of these, three patients presented with brain metastases at the time of study enrolment, and 11 patients developed disease progression with brain metastases during treatment; 19/48 (39.6%) patients had loco-regional disease (Stage III).

Nodular melanoma was the most frequent histopathological subtype (17/48, 35.4%), followed by superficial spreading melanoma (16/48, 33.3%). Median Breslow thickness was 5 mm, and patients were grouped according to this threshold (Table 1). Primary tumour mutational status was as follows: 26 patients (54.2%) had *BRAF* mutant melanoma, three patients (6.2%) had *NRAS* mutant melanoma, and 19 patients (39.6%) were *NRAS* and *BRAF* wild type (Table S1).

Concerning treatment strategies, 29 patients were treated with immunotherapy (28 with anti PD-1, and one with anti PD-1/anti CTLA-4 combination), and 19 patients with target therapy. Anti PD-1 immunotherapy was treatment of choice for NRAS-mutated and BRAF/NRAS wild-type melanoma patients. BRAF-mutated melanoma patients were treated as follows: 19/26 patients with target therapy, 6/26 with anti PD-1 therapy and 1/26 with anti PD-1/anti CTLA-4 combination (Table S1).

3.2 | Plasma ctDNA and patients' characteristics

A total of 177 plasma samples from 48 patients were prospectively collected: plasma collection was performed at the time of treatment initiation and subsequently every 12 weeks, for a mean follow-up of 48.8 weeks (±34.9), (median 38.3 weeks [IQR 20.4–68.2]).

Concerning the 48 baseline plasma samples collected prior to treatment initiation, ctDNA was detected in 16 of 29 patients with known $BRAF^{V600E/K}$- or $NRAS^{Q61R/K}$-mutated primary tumour, yielding a sensitivity of 55.2%. This figure varied depending on disease stage: we obtained a sensitivity of 45.5% (5/11) and 61.1% (11/18) for Stage III and IV patients, respectively. At baseline, the mean plasma ctDNA concentration was 40.3 copies/mL (±137.2), with no significant difference ($p=0.84$) between Stage III (36.4 ± 133.4) and IV (45.0 ± 146.9) disease. Clinical and histopathological features were not significantly different between patients with detectable and non-detectable pretreatment ctDNA (Table 1), except for ulceration that was more frequent in patients with detectable baseline ctDNA ($p=0.003$). CtDNA was not detected in patients with metastasis limited to the central nervous system, both at baseline and upon disease progression.

3.3 | Plasma ctDNA dynamics and tumour response

Plasma ctDNA levels were informative with respect to tumour response assessment: two patients (one Stage IIIB $NRAS^{Q61R}$-mutated melanoma, and one Stage IV BRAF/NRAS wild-type melanoma) with radiologically confirmed disease pseudo-progression, defined as radiologic progression followed by tumour response at subsequent radiological imaging, displayed a favourable plasma ctDNA profile, with undetectable ctDNA copies along a median follow-up of 24 weeks. In addition, one patient with Stage IIIB $NRAS^{Q61K}$-mutated melanoma experienced no extracranial disease, despite brain progression. This patient had high circulating $NRAS^{Q61K}$ ctDNA copies prior to treatment initiation (579.6 copies/mL), which greatly decreased after 12 weeks of nivolumab therapy, and remained undetectable up to 24 weeks. Also, one patient with Stage IIIC BRAF wild-type primary tumour and $BRAF^{V600E}$-mutated inguinal lymph node metastasis presented with high baseline $BRAF^{V600E}$ ctDNA (96 copies/mL), which subsequently declined along with no evidence of disease recurrence during pembrolizumab immunotherapy. Four of the 19 patients with BRAF/NRAS wild-type primary tumour showed disease progression, characterized by a progressive increase in $NRAS^{Q61R}$ ctDNA along longitudinal plasma collection (Table S1).

We found no significant association between baseline plasma ctDNA concentration and the best overall response ($p=0.24$). At the last time point of patients' plasma collection, the average ctDNA concentration significantly differed between responder and non-responder subgroups (0.5 ± 1.7 vs. 160.6 ± 438.3, $p=0.042$), while no significant difference was found according to staging ($p=0.248$).

Investigating plasma ctDNA variation during treatment, we detected a significant increase in plasma ctDNA levels from baseline to the last time point of plasma collection in the non-responder compared to the responder group (median change 3.6 copies/ml [IQR −5.2 to 12] vs. 0.0 copies/ml [IQR −4.2 to 0], $p=0.02$). Logistic regression analysis showed that for each unit increase in the ΔctDNA level, there was a 24% increased risk of disease progression, regardless of treatment type and disease stage at diagnosis (OR = 1.24, 95% CI = 1.03–1.49, $p=0.020$, AUC = 0.932; Table S2; Figure 1A,B). Investigating the mutant ctDNA copies/mL measured at the last time point of patients' plasma collection, we detected a similar trend: for each unit increase in ctDNA concentration, there was a 43% increased risk of disease progression (OR = 1.43, 95% CI = 1.07–1.89, $p=0.014$, AUC = 0.899; Table S2; Figure 1C,D).

3.4 | Plasma ctDNA dynamics and survival outcomes

Baseline ctDNA level as categorical variable (detectable vs. undetectable) was not associated with PFS ($p=0.535$) and OS ($p=0.111$). Conversely, baseline ctDNA level as continuous variable was significantly associated with OS (HR = 1.003, 95% CI = 1.001–1.006, $p=0.043$) and PFS (HR = 1.004, 95% CI = 1.000–1.007, $p=0.029$) as per the multivariable Cox regression analysis, after adjustment for treatment group, age and stage at diagnosis, baseline mutational status and development of brain metastasis during follow-up (Table S3). In addition, higher baseline ctDNA copies/mL showed a trend towards predicting shorter PFS, with a 0.1 decreased week of PFS for each unit increase of baseline ctDNA copies/mL, irrespective of treatment type, baseline mutational status and development of brain metastasis during follow-up (coeff = −0.08, 95% CI = -0.15–0.001, $p=0.053$; Table S4; Figure 2).

Considering ctDNA dynamic variations during treatment, the reduction in ctDNA copies/mL from T0 to Tlast collection was associated with better OS (HR = 0.14; 95% CI = 0.05–0.44, median OS: undefined days vs. 49.7 weeks, p = 0.001) and PFS (HR = 0.08; 95% CI = 0.03–0.27 median PFS: undefined days vs. 30.7 weeks, $p<0.0001$), compared to patients with increased ctDNA level (Figure 3). Following adjustment for clinical confounders, such as type of treatment, number of distant organ metastatic sites, age, mutational status and disease stage at diagnosis, the overtime increase in plasma ctDNA levels from T0 to Tlast collection behaved as an independent predictor of worse survival outcomes, according to the multivariable Cox regression analysis (Table S5).

4 | DISCUSSION

Our results highlight the clinically informative value of ctDNA changes in Stage III and IV CM patients treated with immunotherapy and targeted therapy. We found that higher baseline ctDNA concentration was associated with significantly shorter OS and PFS, independent of treatment group, age and stage at diagnosis, baseline mutational status and occurrence of brain metastasis during treatment. In addition, we observed an association between ctDNA on-treatment variations and patients' survival outcomes, with increased ctDNA concentration from baseline to the last time point of plasma collection being an independent predictor of worse PFS and OS,

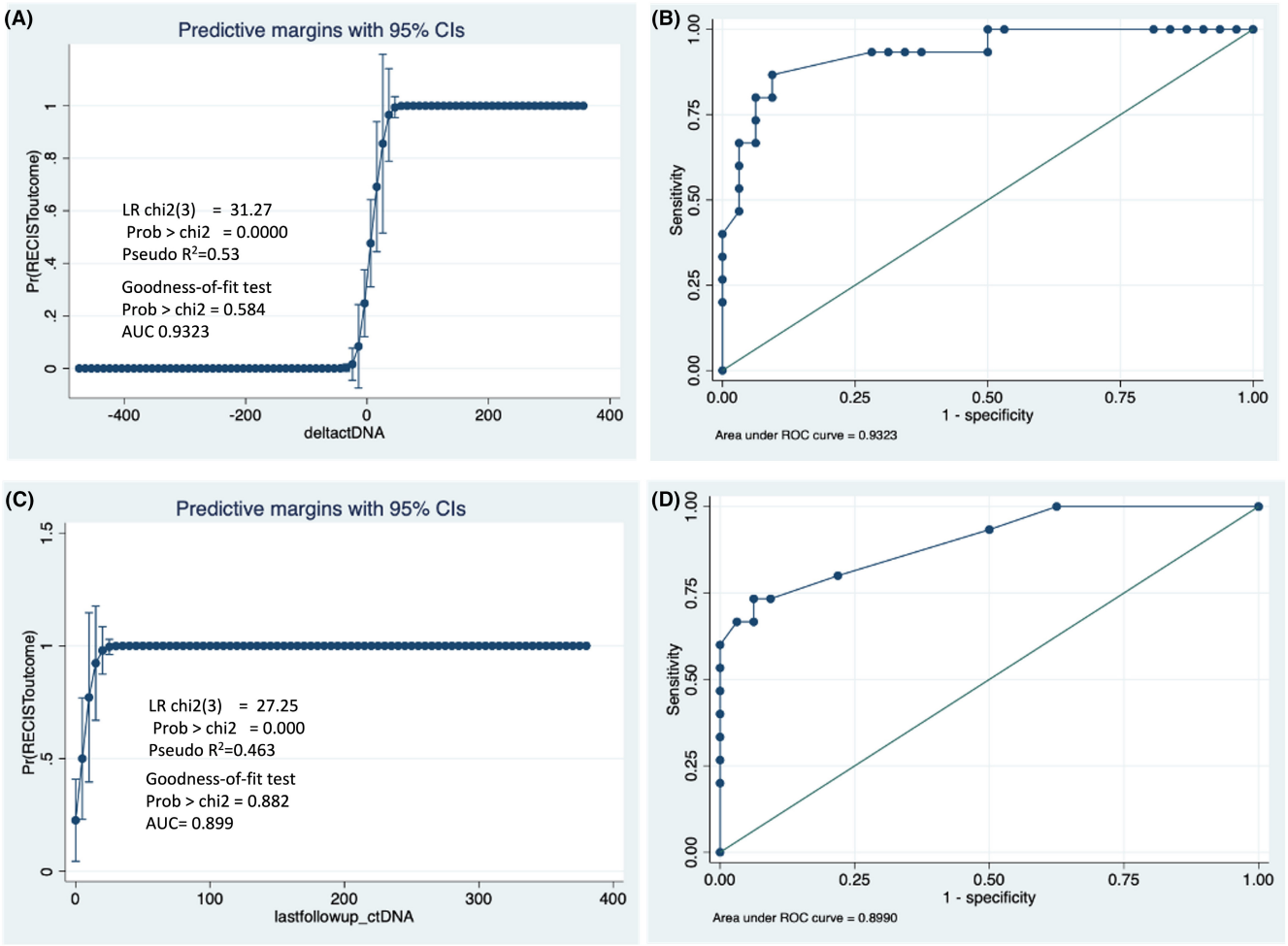

FIGURE 1 Predicted probabilities of tumour response for each level of (A, B) ctDNA variation (ΔctDNA) and (C, D) ctDNA measured at the last time point available for each patients, defining (A, C) the quality of the model (LR) and the goodness-of-fit; (B, D) estimated ability of the model to predict tumour response trough receiver operator characteristic (ROC) curve analysis with area under the curve (AUC).

irrespective of treatment type, number of metastatic sites, age at diagnosis and baseline circulating mutational status. We also found a strong agreement between tumour response and ctDNA dynamics during therapy.

A recent meta-analysis about ctDNA levels and survival outcomes in advanced melanoma patients confirmed the clinical prognostic value of this biomarker,[19] and it provides further evidence about the potential value of ctDNA-based surveillance, irrespective of melanoma stage and the type of systemic therapies.

The best threshold to study quantitative ctDNA changes is yet to be determined: some authors dichotomized between increasing or decreasing ctDNA levels[20,21]; while other investigators distinguished between detectable and zero conversion at Week 4.[17] In our study, we considered baseline pretreatment ctDNA both as a categorical variable (present/absent) and as a continuous variable. We did not find a significant association between ctDNA level (present/absent) and survival outcomes, similarly to recent findings by Syeda et al.[17] Conversely, quantitative ctDNA analysis was significantly associated with OS and PFS, revealing a 0.3% and 0.4% increased risk of death (OS) and disease progression (PFS), respectively, for each unit increase of baseline ctDNA concentration, irrespective of treatment group, age and stage at diagnosis. Plasma ctDNA concentration is known to correlate with tumour burden, therefore its quantitative analysis might be better informative than its qualitative characterization for prediction of patients' clinical outcomes. The optimal timing for plasma ctDNA sampling should also be investigated: Syeda et al.[17] suggested plasma sampling at Week 4 from treatment initiation, whereby undetectable ctDNA levels correlate with improved survival. Nevertheless, early and single time point measurements can lead to false positive results, with high ctDNA concentration due to cancer cell death and consequent ctDNA release.[22] In the present study, we longitudinally sampled plasma ctDNA of target therapy and ICI-treated advanced cutaneous melanoma patients every 3 months, with an average follow-up of 48.8 weeks. CtDNA on-treatment variations were significantly associated with survival outcomes, as well as with tumour response, where we showed that for each unit increase in the ΔctDNA concentration there was a 24% increased risk of disease progression, irrespective of treatment type and disease stage at diagnosis.

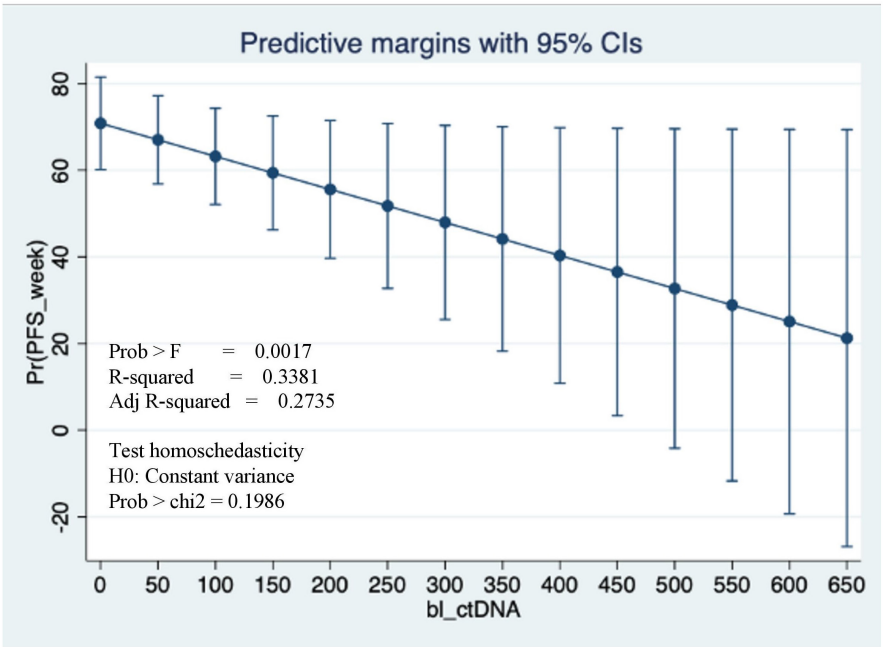

FIGURE 2 Predicted PFS weeks, with 95% confidence interval, according to ctDNA baseline concentration.

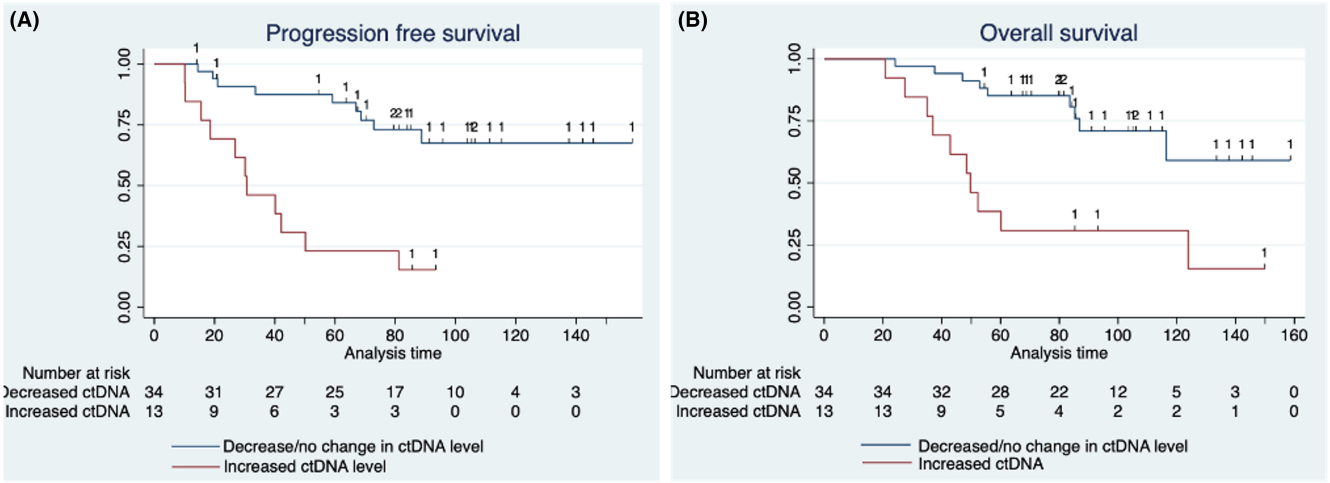

FIGURE 3 Kaplan–Meier plot with censored data for PFS (A) and OS (B), by ctDNA variation during treatment. Cox proportional hazards model showed that the reduction of ctDNA during treatment results in a lower hazard and therefore a longer progression free (C) and overall (D) survivor time.

Plasma ctDNA analysis could also be informative on intra-tumoural, inter-metastatic and temporal heterogeneity. Discrepancies between primary tumour mutational status and metastatic sites have been demonstrated,[23] suggesting the sub-clonality of driver mutations. The analysis of ctDNA might complement all metastatic foci, and provide insights into tumour heterogeneity. In our study, we reported one Stage IIIC patient with BRAF wild-type primitive tumour and $BRAF^{V600E}$-mutated resected inguinal lymph node metastases who exhibited positive baseline ctDNA. The patient was started on adjuvant immunotherapy, during which we observed a ctDNA decline along with no evidence of tumour relapse. Due to occurrence of therapy-related toxicities, the patient had to stop immunotherapy, and was shifted to target therapy, which is currently ongoing. We also reported four patients with Stage IV BRAF/NRAS wild-type primary tumour, who presented with increasing concentration of NRASQ61R-mutated plasma ctDNA along with disease progression during immunotherapy. None of these patients received previous targeted therapy, which could have explained the

acquisition of NRAS mutations. We suggest that temporal heterogeneity should be taken into consideration also for BRAF/NRAS wild-type primary tumour, as recently highlighted by Formica et al. (2021)[24] who evaluated the clinical relevance of tissue/plasma discordant cases in metastatic colorectal cancer patients.

Our analysis has limitations: first, only $BRAF^{V600}$ and $NRAS^{Q61}$ were analysed, while additional variants might improve the accuracy of monitoring strategies. Our study included patients with undetectable mutant ctDNA concentrations at baseline, which however allowed us to not bias the analysis considering that the ctDNA level is associated with tumour burden and worse prognosis. In addition, we were unable to evaluate ctDNA response patterns individually for each therapeutic class because of the small study population, although our findings suggest their applicability across all currently available systemic treatments.

In conclusion, our data highlight the clinically informative value of the dynamic ctDNA changes over time for treatment monitoring of Stage III–IV cutaneous melanoma patients undergoing systemic therapies. The clinical impact of monitoring ctDNA concentrations might be crucial in patients with long-term response, in parallel with scan evaluation, to assess survival outcomes and more accurately evaluate therapeutic efficacy and enable treatment adaptation.

AUTHOR CONTRIBUTIONS

KP, LDN, EC, CA, CP and MCF provided substantial contribution to conception and design. LDR, ADS, MM, BF, SC, LQ, AC and AP contributed to acquisition of data. LDN and KP performed analysis and interpretation of data. LDN, MM, CP, EC, MCF and KP drafted the article and/or revised it critically. All the authors gave final approval of the version to be published.

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CONFLICT OF INTEREST STATEMENT

Alessandro Di Stefani received consulting and speakers fees from Sanofi, Sun Pharma, Pierre Fabre and Galderma. Alessio Cortellini received grant consultancies and speaker fees from MSD, AstraZeneca, EISAI, OncoC4 and IQVIA. Maria Concetta Fargnoli received speaker fees from BMS, MSD and Novartis. Ketty Peris received consulting, lectures, attending meeting and/or travel fees from AbbVie, Almirall, Galderma, Janssen, LEO Pharma, Lilly, Novartis, Pierre Fabre, Sanofi, Sun Pharma, Amgen, MSD and Regeneron.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Supplementary data.

Table S1. Patients' characteristics and ctDNA profile.

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